THE ROLE OF 5-HYDROXYTRYPTAMINE RECEPTORS IN THE REFLEX ACTIVATION OF CARDIAC VAGAL MOTONEURONES IN THE ANAESTHETISED RABBIT AND RAT.

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by

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Abstract.

There is indirect evidence in the literature that activation of 5-HT receptors can modulate vagal control of the heart. The experimental work carried out in this thesis has examined the possibility that cardiac-vagal respiratory responses evoked by stimulating upper airway receptors with smoke, and by the pulmonary C-fibre reflex elicited by i.v. phenylbiguanide (PBG) are modulated by 5-HT pathways. This was done in anaesthetised rabbits and rats by i.c. administration of 5-HT receptor agonists and antagonists. Measurements were made of blood pressure, R-R interval, phrenic nerve activity and renal nerve activity.

In rabbits reflex responses to the above stimuli were inhibited by 5-HT_{1A} antagonists i.c. and potentiated by the 5-HT_{1A} agonist buspirone i.c. Pretreatment with a 5-HT_{1A} antagonist blocked the effect of buspirone. Administration of the 5-HT_{1D} agonist sumatriptan i.c. inhibited the smoke response and the 5-HT_{1D} antagonist GR-127935 i.c. potentiated it. Pretreatment with GR-127935 i.v. blocked the 8-OH-DPAT i.c. inhibition of the smoke response. Administration of a 5-HT_3 antagonist, granisetron i.c. inhibited the response to smoke.

Rats showed a species difference in that 5-HT_{1A} agonists caused an inhibition of the smoke response that could be blocked by pretreatment with a 5-HT_{1A} antagonist, WAY-100802 i.c. and administration of 5-HT_{1A} antagonists i.c. alone did not affect the reflex responses to smoke or PBG in the rat. Administration of granisetron also did not affect the smoke response.

It is therefore concluded that in rabbits activation of central 5-HT_{1A} receptors potentiate reflex activation of cardiac vagal motoneurones whilst these reflexes are inhibited by activation of 5-HT_{1D} receptors. In contrast, central 5-HT_3 receptors exert excitatory control over transmission of the reflexes. There is evidence for a species difference between rabbits and rats.

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Introduction.

Preganglionic cardiac vagal motoneurones

Preganglionic cardiac vagal motoneurones (CVMs) arise from 2 vagal nuclei within the medulla (see Loewy & Spyer, 1990), the nucleus ambiguus (NA) and the dorsal vagal motonucleus (DVMN).

The Nucleus Ambiguus.

The nucleus ambiguus is located in the ventrolateral portion of the medullary reticular formation, extending from just caudal to the facial nucleus to the level of C1 of the cervical spinal cord. The nucleus was named ambiguus because it lacks distinct histological borders. There are 2 divisions to the NA, the ventrolateral division that comprises a column of preganglionic cardiac vagal motoneurones and possibly motoneurones to other viscera and the compact division that comprises a column of motoneurons innervating the upper airways and oesophagus (Bieger & Hopkins, 1987).

Efferent projections.

The neurons of the NA and adjacent reticular formation project to several targets, the nucleus tractus solitarius, cardiac vagal motoneurones, the intermediate grey matter and ventral horn of the spinal cord, the parabrachial nucleus and the facial nucleus (see Loewy & Spyer, 1990).

Afferent projections.

The NA is known to receive afferent input from a number regions of the brain, including the nucleus tractus solitarius, nuclei of the hypothalamus and the medullary reticular formation (see Loewy & Spyer, 1990). Retrograde labelling of cardioinhibitory NA neurones using horseradish peroxidase (HRP) has provided evidence that in the rat the major input to these cells is from the nucleus tractus solitarius (Stuesse & Fish, 1984).

Cardiac vagal motoneurones arise from the NA.

Cardiac vagal motoneurones (CVMs) arise chiefly from the ventrolateral division of the NA. Subepicardial injection of horseradish peroxidase in the cat has produced retrograde labelling of cells in the NA (72 % of cells), the dorsal vagal motonucleus (19%) and the remainder in a zone between the 2 nuclei (Geis et al., 1981). A similar experiment in the rat detected 75 % of CVMs arising from the NA, 8 % from the DVMN and the remainder from an intermediate zone (Izzo et al., 1993).

The Dorsal Vagal Motonucleus.

The dorsal vagal motonucleus (DVMN) is located in the dorsomedial portion of the caudal medulla oblongata, close to the floor of the fourth ventricle. It lies medial to the nucleus tractus solitarius ventrolateral to the area postrema and dorsolateral to the hypoglossal nucleus (Bieger & Hopkins, 1987).

Efferent projections.

About 80 % of the efferent fibres from this nucleus are cholinergic preganglionic vagal fibres (Houser et al., 1983), supplying motor fibres to the heart and gastrointestinal tract from the pharynx to the descending colon (see Loewy & Spyer, 1990). The remaining 20 % of efferent fibres from the DVMN are thought to project centrally to the parabrachial nucleus (King, 1980), the cerebellar cortex and cerebellar nuclei (Zheng et al., 1982).

Afferent projections.

The DVMN receives afferent inputs from both central and peripheral neurones. Central projections arise from a variety of sites in the brain, including the nucleus tractus solitarius, hypothalamic nuclei, medullary reticular formation and raphe obscurus nucleus (see Loewy & Spyer, 1990).

Cardiac vagal motoneurones arise from the DVMN.

The majority of CVMs with B-fibre axons arise from the NA in most species, although rabbits have been found to have substantial B-fibre efferent pathways originating from the DVMN as well as the NA (Jordan et al., 1982). There are a number of lines of evidence to support the view that efferent fibres from the DVMN project to the heart. In the rat DVMN neurones are retrogradely labelled by injection of horseradish peroxidase into cardiac branches of the vagus (Nosaka et al., 1979), neurones with a cardiac rhythm and B fibre conduction velocity have been recorded in the DVMN of the rabbit (Wang et al., 1988) and electrical stimulation of the DVMN (Nosaka et al., 1979), or microinjection of glutamate into the DVMN (Sporton et al., 1991) evokes a bradycardia. Retrograde labelling of cardiac vagal motoneurones by subepicardial injection of horseradish peroxidase has produced the figure of 19 % of cardiac vagal motoneurones located in the DVMN in the cat (Geis et al., 1981) and 8 % in the rat (Izzo et al., 1993).

Extrinsic modulation of CVMs.

There are 2 extrinsic inputs to CVMs that are chiefly responsible for modulating their pattern of activity, the effects of arterial blood pressure and the effects of respiratory drive. The pattern of CVM discharge is maximal 110 - 120 ms after the onset of each pressure wave in the femoral artery, consistent with the latency of the baroreceptor input, evidence that maximal CVM discharge is in phase with the systolic rise in blood pressure due to the excitatory input from the arterial baroreceptors. A respiratory related discharge is superimposed upon this activity which is maximal during post-inspiration. It has been demonstrated using intracellular recording techniques that the respiratory modulation of CVMs is triphasic. During inspiration the CVM is hyperpolarised, the membrane potential then rapidly repolarises at the end of inspiration culminating in depolarisation during post-inspiration. During stage 2 expiration there follows a variable pattern of either depolarisation or hyperpolarisation (Gilbey et al., 1984). It is this pattern of activity that produces the fluctuating heart rate, lowest during early expiration that is termed sinus arrhythmia. The hyperpolarization of CVMs during the inspiratory phase of the respiratory cycle makes them partly or wholly refractory to excitatory inputs from the arterial baroreceptors and chemoreceptors. These excitatory inputs are maximally effective during expiration (Davidson et al., 1976). Stimulation of slowly adapting lung stretch receptors by lung inflation also inhibits these reflexes (Gandevia et al., 1978; Angell-James & Daly, 1978). Excitatory inputs to CVMs also come from arterial baroreceptors, the carotid body, upper airway receptors (see Daly, 1986) and from cardiac and pulmonary C fibres (see Thoren, 1979; see Coleridge & Coleridge, 1984).

The nucleus tractus solitarius.

The nucleus tractus solitarius (NTS) is the major visceral sensory relay of the brain, the initial processing of cardiovascular and respiratory afferent inputs to the central nervous system occurs at this site.

The NTS is located in the dorsal medulla. The nucleus lies ventrolateral to the area postrema and is ventral to the gracile and cuneate nuclei. It forms a Y-shaped column, extending from the level of C1, passing rostrally it bifurcates at the level of opex, to produce a bilateral column of cells extending rostrally to the level of the facial nerve (Thor & Helke, 1987).

Afferent projections.

Fibres from the peripheral chemoreceptors (Miura & Reis, 1972; Lipski et al., 1977), arterial baroreceptors (Lipski et al., 1975), pulmonary stretch receptors (Kalia & Mesulam, 1980), atrial and ventricular receptors (Lee et al., 1972) and sensory fibres from the larynx and pharynx (see Loewy, 1990) all synapse in the nucleus tractus solitarius (NTS). In addition, some sensory fibres from the upper airways and face, running in the trigeminal nerve make their first synapse in the NTS and there are also central projections from the spinal trigeminal nucleus (Menetrey & Basbaum, 1987). The NTS also receives afferent fibres from many central areas, including the raphe nuclei, amygdaloid nucleus, locus coeruleus and paragigantocellular nucleus (see Giersbergen et al., 1992). The fact that the dorsal group of medullary respiratory neurones are also located within the ventrolateral subnucleus of the NTS (see Loewy & Spyer, 1990) has supported the belief that integration of the reflex control of circulation and respiration is achieved through synaptic connections at this level of the brain stem.

Efferent projections.

The NTS sends fibres to a number of areas of the brain thought to be involved in regulation of the cardiovascular system, including the nucleus ambiguus, dorsal vagal motonucleus, caudal raphe nuclei, hypothalamus and locus coeruleus (Loewy & Burton, 1978; Loewy et al., 1986; Thor & Helke, 1987; 1988). These projections appear to be reciprocal and probably function as a feedback system (see Loewy, 1990). The level of activity of NTS neurones determines the signal relayed to these other nuclei involved in cardiovascular regulation. This activity is controlled by the pattern of inputs to the NTS from peripheral cardiovascular receptors and is influenced by more rostral areas involved in the generation of the cardiovascular components of affective behaviour, such as the central nucleus of the amygdala (Cox et al., 1986) and the hypothalamus (Spyer et al., 1986). The reciprocal connections would allow higher nuclei to influence the signal that NTS neurones send to other nuclei, and the observation that NTS efferents do not leave the central nervous system underlines the importance of this nucleus as an integrative relay centre (see Giersbergen et al., 1992).

The NTS and the baroreflex. An example of the role of the NTS in cardiovascular integration.

The role of the NTS in the integration of afferent information is presently best understood in the context of the baroreflex. The NTS receives a rhythmic pattern of input from the arterial baroreceptors, however there is not a one to one relationship between the activity that can be recorded in the carotid sinus or aortic nerves and that recorded from NTS neurones. This may be explained by a combination of factors. First, NTS neurone discharge may be influenced by afferent input from more than one peripheral receptor group (McCall et al., 1977). This is called a spatial interaction, there is evidence both of convergent inputs from different sensory modalities and decussation of input, with fibres from the ipsilateral and contralateral carotid sinus nerve projecting to the same NTS neurone (Felder & Heesch, 1987). An example of spatial interaction is provided by the observation that many NTS neurones excited by baroreceptor inputs also have short latency excitatory input from superior laryngeal nerve afferents (Mifflin et al., 1988a). In addition, different peripheral receptors exhibit variation in threshold and discharge frequency and their afferent fibre conduction velocities vary. Consequently the timing of the arrival of impulses from peripheral receptors results in temporal interaction on NTS unit discharge.

The chemoreceptor reflex.

The main physiological role of the chemoreceptors is regulation of the blood gas composition. The chemoreceptors are divided into 2 groups, the arterial chemoreceptors and the central chemoreceptors. The arterial chemoreceptors are located within the carotid bodies and in the aortic arch and their afferent fibres travel in the sinus nerve and aortic nerve respectively. The aortic chemoreceptors are chiefly oxygen sensors, although they are also sensitive to carbon dioxide tension and pH (see Spyer, 1990). Unmyelinated (Donoghue et al., 1984) and myelinated (Izzo et al., 1988b) fibres of the arterial chemoreceptors project to the nucleus tractus solitarius. The central chemoreceptors are located on the ventral surface of the medulla oblongata and have a specific sensitivity to carbon dioxide tension in arterial blood (see Spyer, 1990). They modulate sympathetic nerve activity through the bulbospinal respiratory pathways (Millhorn, 1986). The chemoreceptors regulate blood gases by modifying respiratory minute volume and cardiac output, particularly in relation to variations in arterial Pa_{O_2} (see Daly, 1983). This is achieved by increasing respiratory rate and depth accompanied by a tachycardia. In order to conserve O_2, stimulation of the chemoreceptors also causes changes in sympathetic vasomotor activity causing vasoconstriction of the vascular beds of the skin, skeletal muscle, heart and kidney, but not the brain (see Daly, 1986). If the chemoreceptors are stimulated in the absence of increased respiratory drive, for instance during the diving response (see below), the O_2 conserving function of the chemoreceptors is particularly pronounced and an intense peripheral vasoconstriction is accompanied by a bradycardia instead of the normal tachycardia. Indeed, denervation of the chemoreceptors virtually abolishes the diving response bradycardia in the duck (Jones & Purves, 1970) and rat (Huang & Peng, 1976).

The diving response.

Stimulation of the nasal mucosa can produce pronounced reflexes affecting respiration, the heart and the peripheral vasculature. These reflexes are subject to variation depending on the strength of stimulus applied to the airway. A mild chemical stimulus may cause an increase in the rate and reduced depth of breathing or sneezing (Allen, 1929; Amdur et al., 1953), whereas stronger irritants can produce apnoea accompanied by hypertension and profound bradycardia or even cardiac arrest (see Daly, 1986). Sudden reflex cardiac arrest may occur during maxillofacial surgery involving any structure innervated by the trigeminal nerve (see Barnard & Bainton, 1990). The pattern of activity comprising apnoea, hypertension and bradycardia is termed the "diving response" by physiologists (Forster & Nyboer, 1955) and the oculocardiac reflex by maxillofacial surgeons (see Barnard & Bainton, 1990). The response can be elicited by a variety of irritants, including, cigarette smoke, ether, sulphur dioxide and distilled water. It has been demonstrated that the afferent pathway of the response is via the trigeminal nerve (Kratchmer, 1870), sensory divisions of which supply the skin of the face and the nasal mucosa. It was found that bilateral section of the trigeminal nerves abolished the response to stimulation of the upper airways whereas section of the olfactory nerves had no effect (Kratchmer, 1870). This conclusion is supported by the finding that mechanical stimulation of the trigeminal nerve results in apnoea, bradycardia and hypertension (see Barnard & Bainton, 1990). The reflex bradycardia and bronchoconstriction are almost entirely dependent on the vagus and are accompanied by inhibition of the phrenic nerve (Brodie & Russell, 1900). However, there is a small cardio-sympathoinhibitory component which can be seen following administration of atropine (see Daly, 1986). The pattern of sympathetic outflow during upper airway stimulation has since been studied and has been demonstrated to be differential, with a renal sympathoexcitation and reduction of activity in the cardiac nerve (Peterson et al., 1983). The sympathoexcitation observed in the renal nerve reflects the generalised peripheral vasoconstriction which occurs during this response, particularly in skeletal muscle as well as the renal and splanchnic vascular beds, with maintenance of blood flow in the common carotid artery (Angell-James & Daly, 1969). The bradycardia and vasoconstriction can be inhibited or even reversed if lung stretch receptors are activated during the response (Angell-James & Daly, 1978).

The central integration of the diving response is complex. The features of the response are a cessation of respiration and maintenance of a preferential blood supply to the brain by peripheral vasoconstriction and bradycardia. As the response persists, hypoxia and hypercapnia progressively develop, stimulating the arterial chemoreceptors (see Daly, 1986). The importance of the chemoreceptors in the maintenance of the bradycardia following immersion of the face in water has been demonstrated in a variety of species. It is virtually abolished by carotid body denervation in the duck (Jones & Purves, 1970) and rat (Huang & Peng, 1976). In the seal, perfusion of the carotid bodies with oxygenated blood early in experimental dives had no effect on the bradycardia, but abolished it later in the dive once hypoxia and hypercapnia had developed. Reperfusion of the carotid bodies with hypoxic blood re-established the bradycardia, but did not elicit a respiratory response (see Daly, 1986) supporting evidence obtained in the dog that stimulation of the carotid body chemoreceptors superimposed upon trigeminal or laryngeal stimulation either fails to elicit any respiratory response or the response is greatly attenuated, but greatly enhances the bradycardia (Daly & Scott, 1958; Angell-James & Daly, 1978). An expiratory apnoea can be maintained in the face of progressively strengthening chemoreceptor drive due to the powerful inhibition of respiratory drive by a trigeminal reflex. The respiratory centres are also less responsive to chemoreceptor afferent stimuli during the expiratory phase of respiration, preventing the carotid body reflex restarting respiration (Black & Torrance, 1971). The trigeminally mediated expiratory apnoea disinhibits cardiac vagal motoneurones (Gilbey et al., 1984), greatly increasing the ability of carotid body chemoreceptor afferents to stimulate the vagus (Angell-James & Daly, 1973), since the CVMs are less refractory to excitatory chemoreceptor input in the absence of central inspiratory drive and input from pulmonary stretch receptors (Gandevia et al., 1978; Gilbey et al., 1984; Daly, 1991). Thus reflexly induced apnoea accompanied by arrest of central inspiratory drive and reduced pulmonary stretch receptor activity allows the unhindered activation of CVMs (Lopes & Palmer, 1976).

The pulmonary C-fibre reflex.

The pulmonary C-fibre reflex comprises a bradycardia, hypotension and apnoea. It is triggered by injection (i.v.) of a variety of substances, including 5-HT, phenylbiguanide (PBG) and veratridine. The pulmonary C-fibre reflex was first described by von Bezold and Hirt in 1867, who demonstrated that the bradycardia was reflex in origin, mediated by the vagus nerve and suggested that it was triggered by the stimulation of receptors in the heart. During experiments with antitoxic serum in the cat, a cardiovascular response similar to that described by von Bezold and Hirt was observed (Brodie, 1900). Investigating the response further, in an effort to determine which vagal fibres were responsible, Brodie demonstrated that denervating the heart abolished the bradycardia, but not the apnoea or hypotension and subsequently found that electrical stimulation of the cut central end of the pulmonary branches of the vagus produced a similar and marked reaction (Brodie & Russell, 1900). The bradycardia of the pulmonary C-fibre reflex is notable because it is not subject to gating by lung stretch receptors (Daly & Kirkman, 1989), or modulation by central respiratory drive (Daly, 1991).

In order to localise the receptors more precisely experiments were performed involving the injection of small quantities of veratrum alkaloids into the vascular supply of the heart and lungs of the dog (Dawes, 1946). It was found that injection of veratridine into the vessels that supply the left ventricle produced the greatest reflex fall in blood pressure and heart rate. Injection of veratridine into the perfused lung also resulted in hypotension and bradycardia and although the pulmonary response was less marked it was accompanied by apnoea. It was therefore concluded that the cardiorespiratory response triggered by injection of various substances was the product of 3 reflexes (see Dawes & Comroe, 1954);

2. A pulmonary depressor reflex causing bradycardia and hypotension.
3. A pulmonary respiratory chemoreflex causing apnoea.

Subsequent studies investigating the origin of afferent cardiac vagal C-fibres in the cat, dog and rat have confirmed the conclusion of Dawes (1946), demonstrating that in the dog and cat, the majority of cardiac vagal afferents arise from the left ventricle (see Thoren, 1979). However, in the rat, no C-fibres have been detected arising from the left ventricle, only from the left atrium with some fibres from the right atrium (Thoren et al., 1979).

Location of central serotonergic nuclei.

The serotonergic system of the mammalian brain is organised into 2 subdivisions, a rostral division and a caudal division. The rostral division has cell bodies in the midbrain and rostral pons and projects mainly to the forebrain. The caudal division is located in the medulla oblongata and has major descending projections, directed chiefly to the spinal cord. Target areas within the brain stem and cerebellum are shared by the 2 divisions. Early in development, the 2 divisions are distinct from each other, however, as growth occurs they move into very close proximity (see Tork, 1990). The cell bodies of serotonergic neurones are located in the midline areas of the caudal midbrain and brain stem and are associated with the raphe nuclei (Dahlstrom and Fuxe, 1965). The development of immunohistochemical techniques, which are more sensitive than immunofluorescent techniques (Steinbusch, 1981) led to the discovery of a more widespread distribution of serotonergic cell bodies and serotonin-containing terminals (Steinbusch, 1981). Lateral to the raphe nuclei, cells extend over the lateral aspect of the inferior olivary nucleus and there are many cells on the ventrolateral surface of the medulla (Hornung & Fritschy,1988; Jacobs et al., 1984). A number of mostly small, bipolar serotonergic neurones are located within the nucleus of the tractus solitarius (NTS; Calza et al., 1985). In man these neurones appear to extend beyond the border of the NTS into the area postrema and reticular formation (Halliday et al., 1988).

Ascending serotonergic projections.

Ascending fibres arise from the rostral division of serotonergic cell bodies, located in the rostral raphe nuclei, the dorsal and median nuclei. Projections from these nuclei travel to the forebrain in 2 main bundles;

1. The dorsal raphe forebrain tract, innervating lateral forebrain structures including the basal ganglia, amygdala, accumbens, thalamus and piriform cortex.
2. The median raphe forebrain tract, innervating medial forebrain structures including the olfactory bulbs, preoptic area, septum, hippocampus and cingulate cortex (Azmitia & Segal, 1978).

There are other ascending tracts from the dorsal raphe nucleus including the cortical tract, passing to the cortex, the periventricular tract, passing to the periventricular region of the thalamus and hypothalamus and the arcuate tract, passing to the substantia nigra, ventrolateral geniculate body nuclei and the suprachiasmatic nucleus of the hypothalamus (Azmitia & Segal, 1978).

Serotonergic innervation of the preoptic nucleus and anterior hypothalamus.

The preoptic nucleus and anterior hypothalamus receive a serotonergic innervation from the dorsal raphe nucleus (Robinson et al., 1985). Microinjection of 5-HT into the region of the preoptic nucleus and anterior hypothalamus in the rat produces a hypertension associated with variable effects on heart rate (Smits & Struycker-Boudier, 1976). There is ample evidence that the 5-HT is mimicking the effect of endogenous 5-HT released from serotonergic terminals originating from the dorsal raphe nucleus. The pressor response to electrical stimulation of the dorsal raphe nucleus is abolished by pretreatment with the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT; Robinson et al., 1985). Furthermore, the response to dorsal raphe nucleus stimulation is prolonged and potentiated by administration of the 5-HT reuptake inhibitor, fluoxetine. Microinjection of 5-HT antagonists into the vicinity of the preoptic nucleus and anterior hypothalamus attenuates the pressor response caused by dorsal raphe nucleus stimulation and the response is abolished by transection rostral to the dorsal raphe nucleus (Kuhn et al., 1980; Robinson et al., 1985; Robinson, 1984). The pressor effect of 5-HT in the preoptic nucleus and anterior hypothalamus is thought to be mediated by 5-HT activating excitatory cholinergic transmission to vasomotor neurones. Central administration of atropine or physostigmine has no effect on resting blood pressure, but results in inhibition of the pressor effect of 5-HT (Krstic et al., 1987; Robinson, 1982).

Descending serotonergic projections.

Descending projections from the caudal raphe nuclei arise mainly from the raphe obscurus, pallidus and magnus and pass to the ventrolateral and dorsal medulla and innervate the dorsal and lateral horns of the spinal cord and the intermediolateral cell column (Dahlstrom & Fuxe, 1964; Loewy, 1981).

Serotonergic innervation of the nucleus ambiguus and dorsal vagal motonucleus.

In the rat and cat, both the NA and DVMN contain neurones immunoreactive for serotonin (Steinbusch, 1981; Izzo et al., 1988) and there is immunohistochemical evidence that serotonergic boutons make synaptic contact with retrogradely labelled cardiac vagal motoneurones in the cat (Izzo et al., 1988a) and rat (Izzo et al., 1993). Autoradiographic studies have demonstrated the presence of 5-HT_{1A} binding sites in the NA and DVMN and 5-HT_2 binding sites in the DVMN (Pazos et al., 1987a; 1987b; Dashwood et al., 1988). Furthermore, microinjection of 5-HT and 8-OH-DPAT into the NA in the cat causes a bradycardia (Izzo et al., 1988a). Microinjection of 8-OH-DPAT into the DVMN in the rat also evokes a bradycardia (Sporton et al., 1991).

Serotonergic innervation of the nucleus tractus solitarius.

The nucleus tractus solitarius (NTS) has been found to contain virtually every neurotransmitter and neuromodulator known to occur in the central nervous system (see Giersbergen et al., 1992). It has been demonstrated that serotonergic neurones project to this nucleus from the vagus nerve (Gaudin-Chazal et al., 1982) and the raphe nuclei (Thor & Helke, 1987). Microinjection of 5-HT into the NTS elicits a reduction in mean arterial blood pressure, heart rate and renal nerve activity, which could be inhibited by

$5\text{-}HT_{1A}$ and to a lesser extent $5\text{-}HT_2$ receptor antagonists (Itoh & Bunag, 1990). Electrophysiological studies have demonstrated that exogenously applied 5-HT causes depression of the spontaneous activity of individual NTS neurones, probably through activation of $5\text{-}HT_{1B}$ receptors (Feldman, 1994).

Serotonergic innervation of the nucleus paragigantocellularis lateralis.

The nucleus paragigantocellularis lateralis (PGL) receives a relatively dense 5-HT innervation (Steinbusch, 1981). This area of the ventrolateral medulla is thought to be of importance in the maintenance of blood pressure. If the surface of the rostral ventrolateral medulla is lesioned large falls in blood pressure occur (Guertzenstein & Silver, 1974). Conversely, electrical stimulation of this region results in a pressor response and the effect is blocked by lesioning serotonergic neurones with the neurotoxin 5,7-DHT (Dampney et al., 1982; Howe et al., 1983). Administration of D,L-homocysteic acid (DLH) to selectively activate neuronal perikarya produced the same effect, proving that the responses are not solely due to activation of fibres of passage (Dampney et al., 1982). Bilateral microinjection of 5-HT into this area produces a fall in blood pressure associated with a bradycardia (Lovick, 1988).

Serotonin receptors.

There are multiple 5-HT receptor types and the effect of exogenous 5-HT therefore depends on which type of receptor is being stimulated. 5-HT receptors were initially divided into 2 groups, M and D type receptors on the basis of 5-HT receptor antagonist effects on guinea pig ileum (Gaddum & Picarelli, 1957). Subsequent research used radioligand binding in rat brain membranes to identify 5-HT acceptor sites. At this point it is important to distinguish between receptors, to which a neurotransmitter or drug can bind to cause a response and acceptors, to which a ligand can bind without eliciting a response, such as carrier proteins and intracellular binding proteins. Evidence for a high and a low affinity binding site for 5-HT was found, but the pharmacology of these sites differed from those identified by Gaddum & Picarelli. They were labelled $5\text{-}HT_1$ and $5\text{-}HT_2$ receptors for the high and low affinity sites respectively (Peroutka & Snyder, 1979).

This research led to the numbered system of classification for 5-HT receptors used presently. Initially, 3 principle 5-HT receptor types were recognised on the basis of pharmacological differences and were named "5-HT_1-like", 5-HT_2 and 5-HT_3 receptors (Bradley et al., 1986). The advent of molecular biological techniques allowed the structure of proteins to be deduced from the sequence of their gene. This has resulted in the identification of multiple receptor types for 5-HT and molecular biology is now the cardinal technique employed to define receptor types, although not every 5-HT receptor gene has been found at the present time (e.g. 5-HT_4). The 5-HT receptors are now grouped into 7 families (see Hoyer et al., 1994). Each family contains receptor subtypes related by their similar molecular biological, pharmacological and biochemical properties.

The 5-HT receptor types of particular interest when considering the modulation of cardiovascular reflexes are the 5-HT_{1A}, 5-HT_{1B} or 5-$HT_{1D\beta}$ in non-rodent species, 5-$HT_{1D\alpha}$, 5-HT_2 and 5-HT_3 receptors.

The 5-HT_1 receptor class.

The term "5-HT_1-like" receptor was proposed for the heterogeneous group of receptors exhibiting high affinity for 5-HT and its close analogue, 5-carboxamido-tryptamine (5-CT). The pharmacological criteria used to define this receptor class by Bradley et al., have been superceded now that molecular biological techniques have allowed the receptors to be defined using their genetic sequences.

All 5-HT_1 receptors characterised so far are seven transmembrane domain receptors which are negatively coupled to adenylyl cyclase via a regulatory G-protein. The 5-HT_1 receptor class is now divided into 5 sub-types, 1_A, 1_B, 1_D, 1_E and 1_F.

5-HT_{1A} receptors.

From a cardiovascular perspective, activation of central 5-HT_{1A} receptors predominantly results in sympathoinhibition and increased cardiac vagal motor tone (see McCall & Clement, 1994). Administration i.v. of the archetypal 5-HT_{1A} agonist, 8-OH-DPAT, has been demonstrated to cause hypotension and bradycardia in rabbits (Hof & Fozard, 1989; Shepheard et al., 1990a), cats (Ramage & Fozard, 1987, McCall et al., 1987; Doods et al., 1988) and rats (Gradin et al., 1985; Fozard et al, 1987). The fall in blood pressure has been shown to be due to a reduction in sympathetic nerve activity in rabbits (Shepheard et al., 1990a) and cats (Ramage & Fozard, 1987; McCall et al., 1987). The evidence that the hypotension and sympathoinhibition is due to a central site of action is as follows. It was demonstrated in the cat that administration of 8-OH-DPAT (i.v.) caused a hypotension, sympathoinhibition and bradycardia, despite a reduction in baroreceptor afferent activity (Ramage & Fozard, 1987) and administration of 8-OH-DPAT (i.v.) will cause a sympathoinhibition even if the cat is baroreceptor denervated (McCall et al., 1987). Furthermore, the effect of a particular dose of 8-OH-DPAT or flesinoxan administered either into the vertebral artery or intracisternally (i.c.) is greater than the same dose given intravenously (i.v.) (Doods et al., 1988; Wouters et al., 1988). If the animal is pithed before administration of the agonist, no fall in heart rate or blood pressure is seen, even if basal blood pressure is maintained with angiotensin II (Fozard et al, 1987) and the bradycardia can be blocked by pretreatment with atropine and propranolol (Gradin et al., 1985).

5-HT$_{1A}$ receptor mediated activation of cardiac vagal motoneurones.

The observations that *d*-lysergic acid diethylamide, an agonist with high affinity for all 5-HT$_1$ receptor types, increased cardiac vagal tone in decerebrate cats (Cervoni et al., 1963) and that administration of 5-hydroxytryptophan, a precursor of 5-HT caused a significant fall in baseline heart rate and an inhibition of reflex bradycardia in spinalised, anaesthetised cats (Tadepalli, 1980) led to the hypothesis that 5-HT may be important in the modulation of cardiac vagal drive. It has been demonstrated that serotonergic neurones make synaptic contact with retrogradely labelled cardiac vagal motoneurones (Izzo et al., 1988a; 1993) and that administration of 5-HT$_{1A}$ receptor agonists causes an increase in cardiac vagal motoneurone tone in the cat (Izzo et al., 1988a) and rat (Cherqui et al., 1988). The bradycardia can be inhibited by vagotomy or the administration of atropine (Gradin et al., 1985; Ramage & Fozard, 1987; Ramage et al., 1988). Both the nucleus ambiguus (NA) and the dorsal vagal motonucleus (DVMN) receive a strong serotonergic innervation (Steinbusch, 1981) and microinjection of 5-HT into the NA of the cat elicits a vagal bradycardia, which is mimicked by microinjection of 8-OH-DPAT (Izzo et al., 1988a). In the rat, microinjection of the 5-HT_{1A} receptor agonists, 8-OH-DPAT and flesinoxan into the DVMN elicit a decrease in heart rate (Sporton et al., 1991). Furthermore, it has been demonstrated that 5-HT_{1A} receptors play an excitatory role in the modulation of the reflex bradycardia elicited during the pulmonary C-fibre reflex triggered by i.v. phenylbiguanide (PBG) in the rat (Bogle et al., 1990).

$5\text{-}HT_{1A}$ receptor ligands.

There are a number of strands of evidence to support 5-HT_{1A} receptors as the site of action for 8-OH-DPAT and flesinoxan. First of all, the effects can be mimicked by a variety of compounds which are structurally diverse but share 5-HT_{1A} receptor agonism as a common property. These include buspirone (Romero et al., 1993), ipsapirone (Ramage & Fozard, 1987), urapidil (Ramage, 1986) and U-92016A (Romero et al., 1993). In addition, the cardiovascular effects of 8-OH-DPAT can be prevented by administration of 5-HT_{1A} receptor antagonists such as (-)-pindolol, metergoline and methiothepin (Fozard et al., 1987; Doods et al., 1988) or WAY-100135 (Escandon et al., 1994), yet these compounds have no effect on the central sympathoinhibitory effects of the α_2-receptor agonist, clonidine. The 5-HT_2 receptor antagonist, ketanserin, the 5-HT_3 receptor antagonist MDL-72222 and the α_2-receptor antagonist idazoxan all fail to antagonise the cardiovascular effects of 8-OH-DPAT (Fozard et al., 1987; Ramage & Fozard, 1987). Understanding of the function of 5-HT_{1A} receptors will be helped by the recent development of a 5-HT_{1A} antagonist, WAY-100635, an achiral analogue of WAY-100135. This drug has high affinity for 5-HT_{1A} receptors ($pA_2 = 9.71$) and, unlike WAY-100135, does not depress the firing rate of dorsal raphe neurones at doses up to 100 $\mu g\ kg^{-1}$ i.v. (Forster et al., 1995).

Distribution of 5-HT_{1A} receptors.

The distribution of 5-HT_{1A} receptors within the CNS has been demonstrated in a variety of species using autoradiographic techniques. They are densely localized in the dorsal and median raphe nuclei (Marcinkiewicz et al., 1984; Radja et al., 1991; Thor et al., 1992a) and in the limbic system (hippocampus, septum, amygdala and cortical limbic area; Hoyer et al., 1986a; Pazos et al., 1987a). Autoradiographic mapping techniques have located high concentrations of 5-HT_{1A} binding sites in the NA, DVMN, NTS and the nucleus of the spinal tract of the trigeminal nerve of the rat brain (Pazos & Palacios, 1985; Dashwood et al., 1988; Manaker & Verderame, 1990; Thor et al., 1992b). It is known that 5-HT_{1A} receptors function both as autoreceptors, modulating serotonergic neurones as well as heteroreceptors, located on non-serotonergic neurones. This was demonstrated using the serotonergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), administration of which reduced the density of 5-HT_{1A} binding sites located on serotonergic neurones found in the raphe nuclei by destruction of the neurones bearing somatodendritic 5-HT_{1A} autoreceptors (Weissman-Nanopoulos et al., 1985), but leaving the density of 5-HT_{1A} heteroreceptors located on non-serotonergic neurones unchanged (Verge et al., 1986).

5-HT_{1B} receptors.

Many studies have determined that terminal autoreceptors of the rat cortex are of the 5-HT_{1B} receptor subtype and that there is a highly significant correlation between the potencies of drugs for the rat autoreceptors and their affinities for 5-HT_{1B} binding sites (Middlemiss, 1984; 1985; Engel et al., 1986; Limberger et al., 1991). It has been demonstrated that 5-HT_{1B} receptors function as presynaptic autoreceptors, inhibiting the release of 5-HT (Middlemiss, 1986; Limberger et al., 1991). There is also ample evidence that 5-HT_{1B} receptors function as terminal heteroreceptors to control the release of other neurotransmitters, such as acetylcholine and glutamate (Engel et al., 1986; Maura & Raiteri, 1986; Raiteri et al., 1986). Heteroreceptors may even predominate since lesions of serotonergic neurones do not produce significant reductions in 5-HT_{1B} binding in most areas examined (Thor et al., 1992a).

There are a number of ligands said to have selectivity for the 5-HT_{1B} receptor, including, TFMPP, mCPP and CGS-12066. However, none of these ligands displays more than a 3 fold selectivity for the 5-HT_{1B} receptor over other 5-HT receptor subtypes (Schoeffter & Hoyer, 1989a). No good selective antagonists are available at this time. Some β-adrenoceptor antagonists, such as pindolol, have potent 5-HT_{1B} receptor antagonist properties, but have similar affinity for 5-HT_{1A} receptors.

The 5-HT_{1B} receptor subtype is found in abundance in the basal ganglia of rats and mice, especially in the globus pallidus, and the pars reticulata of the substantia nigra (Pazos and Palacios, 1985) significant densities of 5-HT_{1B} receptors have also been detected in the trigeminal nucleus, nucleus tractus solitarius and dorsal vagal motonucleus (Manaker & Verderame, 1990; Thor et al., 1992a; 1992b). These binding sites correspond to the $5\text{-HT}_{1D\beta}$ receptor in other mammalian brains (Oskenberg et al., 1992). 5-HT_{1B} receptor mRNA has also been reported in raphe nuclei, striatum, cerebellum, hippocampus, entorhinal and cingulate cortex, subthalamic nucleus and nucleus accumbens (Voigt et al., 1991; Maroteaux et al., 1992; Jin et al., 1992).

The pharmacological differences between the 5-HT_{1B} and 5-HT_{1D} receptor are apparently due to the substitution of an asparagine residue at position 351 of the rat 5-HT_{1B} receptor gene for a threonine residue at this position in the human $5\text{-HT}_{1D\alpha}$ and $5\text{-HT}_{1D\beta}$ receptor genes. It is thought that this single naturally occurring "mutation" accounts for the pharmacological differences between rat 5-HT_{1B} receptors and non-rodent 5-HT_{1D} receptors (Adham et al., 1992).

5-HT_{1D} receptors.

The 5-HT_{1D} receptor subtype has been located in a range of mammalian species including guinea-pig, hamster, rabbit, dog, pig, calf and human (Heuring & Peroutka, 1987; Hoyer & Schoeffter, 1988; Herrick-Davies & Titeler, 1988; Beer et al., 1992; Maura et al., 1993). 5-HT_{1B} receptors appear to be absent in these species and the 5-HT_{1D} receptor distribution reflects that of the 5-HT_{1B} receptor found in rodents. There is evidence, however, that 5-$HT_{1D\alpha}$ receptors are present in the rat brain (Herrick-Davis & Titeler, 1988; Bruinvels et al., 1993b; Davidson & Stamford, 1995). The function of 5-HT_{1D} receptors has been demonstrated to be very similar to that of 5-HT_{1B} receptors. The role of the 5-HT_{1D} receptor was identified as the mediation of inhibition of 5-HT release from cortical nerve terminals of the guinea pig brain (Middlemiss et al., 1988). It was subsequently demonstrated that the potencies of a variety of agonists and antagonists at the 5-HT_{1D} receptor mediating inhibition of adenylyl cyclase correlated with their effects on release of [^{3}H]5-HT in pig cortex slices (Schlicker et al., 1989). These studies strongly suggest that the terminal 5-HT autoreceptor is of the 5-HT_{1D} subtype in non-rodent mammalian species.

5-HT_{1D} receptors are also thought to function as heteroreceptors. 5-HT inhibits release of glutamate from rat cerebellar synaptosomes (Raiteri et al., 1986) and acetylcholine from guinea pig hippocampal synaptosomes (Harel-Dupas et al., 1991).

Few selective ligands for the 5-HT_{1D} receptor have yet been discovered, and many drugs "selective" for other 5-HT receptor subtypes have appreciable affinity for these receptors, such as 8-OH-DPAT (pK_D=6.9; Bruinvels et al., 1992) and nn-DP-5-CT (pK_D=6.6; see Hoyer & Fozard, 1991). Sumatriptan is one agonist possessing limited selectivity for this subtype (pK_D=6.3 at 5-HT_{1D} receptors, pK_D=5.6 at 5-HT_{1A} receptors; Peroutka & McCarthy, 1989; Schoeffter & Hoyer, 1989b). A very selective and potent 5-HT_{1D} receptor antagonist, GR-127935 has recently been synthesised, (pK_D=8.5; Skingle et al., 1993).

The regional distribution of 5-HT_{1D} receptors in non-rodent species appears very similar to that of 5-HT_{1B} receptors in the rat, with the highest densities located in the substantia nigra, basal ganglia and nigrostriatal pathway. Lower densities are found in the raphe nuclei, hippocampus and cortex (Waeber et al., 1990). In all species the density of

$5\text{-}HT_{1D\alpha}$ receptor mRNA is much lower than that of $5\text{-}HT_{1D\beta}$ receptors and there is similar distribution of the 2 receptor subtypes (Hamblin et al., 1992; Bach et al., 1993).

$5\text{-}HT_{1E}$ receptors.

The $5\text{-}HT_{1E}$ receptor was first identified in homogenates of human frontal cortex using binding studies with [^{3}H]5-HT in the presence of excess 5-CT to block $5\text{-}HT_{1A}$ and $5\text{-}HT_{1D}$ binding (Leonhardt et al., 1989). Homogenate binding studies indicate that the $5\text{-}HT_{1E}$ receptor has a similar distribution to the $5\text{-}HT_{1D}$ receptor and now that the $5\text{-}HT_{1E}$ receptor gene has been cloned, the distribution should be confirmed using *in situ* hybridisation techniques to locate the receptor's mRNA.

The *in vivo* function of the $5\text{-}HT_{1E}$ receptor is not known at present, but *in vitro* experiments performed using $5\text{-}HT_{1E}$ receptor-transfected cells demonstrated that it is negatively coupled to adenylyl cyclase (McAllister et al., 1992), justifying its inclusion in the $5\text{-}HT_1$ receptor group. The development of an understanding of the *in vivo* function of this receptor is hindered by the absence of any selective $5\text{-}HT_{1E}$ receptor ligands (see Humphrey et al., 1993).

$5\text{-}HT_{1F}$ receptors.

The mRNA of the human $5\text{-}HT_{1F}$ receptor has been identified in the brain, mesentery and uterus, with the mRNA in the brain concentrated in the dorsal raphe, hippocampus and cortex (Adham et al., 1993a; Adham et al., 1993b). $5\text{-}HT_{1F}$ receptors have also been found in the mouse, located in the cortex, striatum and hippocampus (Amlaiky et al., 1992).

The $5\text{-}HT_{1F}$ receptor is negatively coupled to adenylyl cyclase in cells transfected with a $5\text{-}HT_{1F}$ receptor clone (Adham et al., 1993b, Amlaiky et al., 1992). Nothing is known about the role of $5\text{-}HT_{1F}$ receptors *in vivo* although their distribution is said to suggest a role as another 5-HT autoreceptor type (Adham et al., 1993a). There are presently no selective agonists or antagonists available for this receptor subtype.

The 5-HT_2 receptor class.

The 5-HT_2 receptor class was discovered by Gaddum & Picarelli, who labelled it the D receptor. The D receptor was subsequently renamed a 5-HT_2 receptor (Humphrey et al., 1982). At present 3 subtypes of the 5-HT_2 receptor are recognised (5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}). Since this classification has only recently been made and no ligands are available that can discriminate between the 3 receptor subtypes, the main body of literature discusses the role of 5-HT_2 (or 5-$HT_{2/1C}$) receptors as a single group.

5-HT_{2A} receptors are located both centrally and peripherally, chiefly on smooth muscle but also functioning to regulate platelet aggregation and capillary permeability (Bradley et al., 1986), 5-HT_{2B} receptors are located in the stomach fundus and 5-HT_{2C} receptors are especially abundant in the choroid plexus. Each receptor subtype has been cloned and shown to be G-protein linked, with seven trans-membrane domains, mediating its effects by activation of phosphoinositide metabolism.

It has been demonstrated that activation of 5-HT_2 receptors produces opposing effects to activation of 5-HT_{1A} receptors on autonomic outflow (Shepheard et al., 1991). In the cat administration of 5-HT_2 receptor agonists results in a centrally mediated sympathoexcitation. An intravenous dose of the 5-HT_2 receptor agonists DOI or quipazine elicits an increase in blood pressure, mediated partly by a peripheral vasoconstrictor action, accompanied by a sympathoexcitation (McCall et al., 1987; Vayssettes-Courchay et al., 1991; Ramage et al., 1991). The sympathoexcitation can be reversed by 5-HT_2 receptor antagonists such as ketanserin or LY-53857 and is blocked by pretreatment with these drugs (McCall & Harris, 1988). The 5-HT_2 receptors mediating these effects are possibly located at the level of the sympathetic preganglionic neurone, since it has been demonstrated that these neurones receive an excitatory input from medullospinal serotonergic neurones (McCall, 1983; 1984). However it has been found that although microiontopheretic application of 5-HT excites these neurones, application of DOI does not influence their firing rate (Clement & McCall, 1990). This observation may perhaps reflect a 5-HT_2 receptor site of action located on distal dendrites of these neurones, but the precise role of the rostral ventrolateral medulla in the sympathoexcitatory action of 5-HT_2 receptor agonists remains to be determined. In addition to these effects on sympathetic outflow, 5-HT_2 receptor agonists have been found to decrease the rate of central inspiratory drive (Shepheard et al., 1991). 5-HT_2 receptors have also been implicated in the control of release of β-endorphin, corticosterone (Cohen et al., 1983), renin (Rittenhouse et al., 1991; Bagdy et al., 1992) and prolactin (Van der Kar, 1991).

Drugs which can be used as selective 5-HT_2 receptor agonists include the hallucinogenic agents DOI, DOM and DOB (Shannon et al., 1984; Glennon et al., 1986; 1988; Titeler et al., 1985) and α-methyl-5-HT (Feniuk, et al., 1985; Dalton et al., 1986). However, all these compounds also have high affinity for the 5-HT_{2C} receptor in addition to their action at 5-HT_{2A} receptors. The first potent 5-HT_{2A} receptor antagonist to be described was ketanserin, however, this drug also has affinity for 5-HT_{2C} receptors and is not selective with respect to other neurotransmitters, binding to α_1-adrenoceptors and histamine receptors (Leysen et al., 1981; 1982; Van Nueten et al., 1981). Several other 5-HT_{2A}/5-HT_{2C} receptor antagonists have been discovered including mesulergine, LY-53857, cinanserin and ritanserin (Cohen et al., 1983; Rubin et al., 1964; Leysen et al., 1985).

It has been known for many years that the rat fundic strip is very sensitive to 5-HT (Vane, 1959). However the 5-HT receptor responsible for fundic smooth muscle contraction proved very difficult to characterise. Although the fundus receptor shared some characteristics with the classical 5-HT_2 receptor, on the basis of agonist and antagonist dissociation constants it was clearly not a 5-HT_{2A} receptor (Cohen & Wittenauer, 1987). The fundus receptor was shown to bear a resemblance to the 5-HT_{2C} receptor (then classified as 5-HT_{1C} receptor; see below) on the basis of the rank order of a variety of agonists (Buchheit et al., 1986). However, it was subsequently demonstrated that 5-HT_{2C} receptor mRNA is not present in rat fundus preparations

(Baez et al., 1990; Foguet, et al., 1992). The receptor was eventually cloned and named the 5-HT_{2F} (fundus) receptor (Kursar et al., 1992). This receptor has since been renamed the 5-HT_{2B} receptor (see Humphrey et al., 1993).

Early autoradiographic studies performed using 5-HT_1 receptor ligands such as [^{3}H]LSD and [^{3}H]5-HT demonstrated the presence of high densities of binding sites in the choroid plexus. These sites were not labelled with 5-HT_2 receptor ligands, except [^{3}H]mesulergine (Meibach et al., 1980; Pazos et al., 1985). It was therefore assumed that these receptors belonged to the 5-HT_1 class and were named 5-HT_{1C} (Pazos et al., 1985). However, further investigation into the nature of the 5-HT_{1C} receptor revealed its close structural homology with the 5-HT_{2A} receptor, together with a shared second messenger transduction system, that is, activation of phospholipase C (Julius et al., 1988), and very similar operational characteristics. This clearly indicated that the receptor should be reclassified as a 5-HT_2 receptor subtype and it is now labelled the 5-HT_{2C} receptor (see Hoyer, 1988; see Hartig, 1989).

The 5-HT_3 receptor.

The 5-HT_3 receptor was discovered by Rocha e Silva et al., (1953) who demonstrated the ability of low concentrations of cocaine to block 5-HT mediated contractions of guinea-pig ileum, without affecting the responses to pilocarpine or histamine. The receptor responsible for this observation was labelled the M receptor by Gaddum and Picarelli (1957). It was renamed the 5-HT_3 receptor by Bradley et al. (1986).

The 5-HT_3 receptor is associated exclusively with neurones of both peripheral and central origin (see Fozard, 1992). In the brain, the highest densities of 5-HT_3 receptors are found in discrete nuclei of the lower brain stem, including the dorsal vagal motor nucleus, nucleus tractus solitarius (NTS), spinal trigeminal nucleus, the area postrema and substantia gelatinosa (Hamon, et al., 1989; Pratt et al., 1990). Lower, but still significant densities of 5-HT_3 receptors are located in the cortex and areas of the limbic system such as the hippocampal formation, amygdala and medial nucleus of the habenula

(Kilpatrick et al., 1987; Waeber et al., 1988; Kilpatrick et al., 1990). The 5-HT_3 receptors identified in the nucleus tractus solitarius are thought to be located presynaptically on vagal afferent terminals, since nodose ganglionectomy results in a 50% reduction in binding of 5-HT_3 receptor ligands in the NTS (Pratt & Bowery, 1989). In the periphery, 5-HT_3 receptors are located on pre- and postganglionic neurones of the autonomic nervous system and on neurones of the sensory and enteric nervous systems (Fozard, 1984a; Hoyer et al., 1989b).

The 5-HT_3 receptor is a ligand gated cation-selective channel, activation of which results in a rapid depolarisation of the neurone (Derkach et al., 1989). 5-HT_3 receptors desensitise and resensitise rapidly (Yakel et al., 1991) using both cAMP-dependent and cAMP-independent mechanisms. The major consequence of a 5-HT_3 receptor mediated depolarisation of the neurone is activation of voltage-gated Ca^{2+} channels, producing a rapid increase in the cytosolic Ca^{2+} concentration. Many of the functions of 5-HT_3 receptors are concerned with the modulation of neurotransmitter release. This has been demonstrated both in peripheral tissues such as the acetylcholine-substance P mediated contraction of guinea-pig ileum (Fox et al., 1989) and in the central nervous system, both for dopamine (Blandina et al., 1988) and for acetylcholine (Barnes et al., 1989). Activation of 5-HT_3 receptors has profound effects on many physiological systems studied in vivo. Dramatic responses may be triggered in the cardiovascular system, the heart rate and contractility may be inhibited or excited by reflex and local pathways (see Saxena & Villalon, 1991). 5-HT_3 receptors are involved in the transmission of pain and sensitisation of nociceptive neurones in the sensory nervous system (Richardson et al., 1985; Hamon et al., 1989).

A number of 5-HT_3 receptor agonists are known, including, 2-methyl-5-HT, phenylbiguanide and m-chlorophenylbiguanide. Of these drugs, the latter is the most potent and is more selective than 2-methyl-5-HT, which also has appreciable affinity for 5-HT_1 receptors (Kilpatrick et al., 1990; Tadipatri et al., 1992). All of these agents have the disadvantage of being partial rather than full agonists at the 5-HT_3 receptor (Ireland

& Tyers, 1987; Sepulveda et al., 1991). There are now many compounds available that exhibit antagonist effects at the 5-HT_3 receptor. The most thoroughly studied are MDL-72222 (Fozard, 1984b), tropisetron (Richardson et al., 1985), ondansetron (Butler et al., 1988) and granisetron (Sanger & Nelson, 1989). At this stage there is no strong evidence for the existence of intraspecies 5-HT_3 receptor subtypes, although a recent report demonstrated differences between affinities of ligands for 5-HT_3 recognition sites in membranes from 2 mouse tissues, cortex and ileum (Bonhaus et al., 1993). This suggests the possible existence of 5-HT_3 receptor subtypes, but formal recognition awaits the development of more selective ligands and details of the structure of the receptor proteins.

Peripheral 5-HT_3 receptors mediate the pulmonary C-fibre reflex.

The observations that the pulmonary C-fibre reflex could be triggered both by 5-HT and PBG, that the isolated rat vagus nerve was equipotently depolarised by both agonists and that both effects of both drugs could be antagonised by metoclopramide led to the suggestion that the drugs were acting through the same 5-HT receptor subtype (Fortune et al., 1983; Collins & Fortune, 1983). It was subsequently demonstrated that the pulmonary C-fibre reflex caused by 5-HT in the anaesthetised and conscious rat could be inhibited by the 5-HT_3 receptor antagonist MDL-72222. It is thought that the 5-HT_3 receptors are located on vagal sensory neurones (Ireland & Tyers, 1987; Veelken et al., 1990). A similar chemoreflex can be elicited by intrapericardial nicotine. This response is not affected by MDL-72222, suggesting that nicotine and serotonin trigger similar reflex responses in epicardial vagal afferent fibres by stimulating different epicardial receptors (Veelken et al., 1990).

The 5-HT_4 receptor.

The 5-HT_4 receptor is a 7 transmembrane domain, G-protein coupled receptor positively linked to adenylyl cyclase. The intracellular responses to activation of this receptor are elicited by elevated cAMP levels and consistent with this observation, 5-HT_4 receptors appear to mediate excitatory events such as increased transmitter release and increased myocardial contractility. No cDNA clone has yet been described for the 5-HT$_4$ receptor and the identification and characterisation of this receptor is presently based largely on its desensitisation through phosphorylation by a specific agonist-dependent receptor kinase (Dumuis et al., 1989; Craig et al., 1990). The 5-HT$_4$ receptor was first described in mouse and guinea pig brain (Dumuis et al., 1988). It is now known to be distributed widely throughout the brain and periphery of a variety of species including human (Kaumann et al., 1989) and pig (Bom et al., 1988; Kaumann et al., 1990). The distribution of 5-HT$_4$ receptors suggests that they may be involved in affective disorders, psychoses, motor co-ordination, arousal, visual perception, learning and memory. In the periphery, 5-HT$_4$ receptors have been identified on neurones (Craig & Clarke, 1990), smooth muscle cells (Bieger & Triggle, 1985; Baxter et al., 1991) and secretory cells (Bunce et al., 1991) in the alimentary tract. They are also located in the atria of a number of species including man. In the denervated heart, 5-HT$_4$ receptor activation evokes a tachycardia in isolated, spontaneously beating piglet right atrium (Kaumann, 1990) and a positive inotropic effect on isolated left atrium (Kaumann et al., 1990). 5-HT$_4$ receptors have been implicated in the development of atrial arrhythmias (Kaumann, 1994).

5-HT$_4$ receptor ligands fall into one of three categories, 5-HT and related indoles, substituted benzamides and azabicycloalkyl benzamidazolones. Two examples of benzamide 5-HT$_4$ receptor agonists are renzapride and cisapride, the efficacy and potency of these drugs is highly species dependent. A number of potent and selective 5-HT$_4$ receptor antagonists have recently been developed. GR-113808A and SB-207710 are both particularly potent and have over 1000 fold greater affinity for 5-HT$_4$ receptors over 5-HT$_3$ receptors (Kaumann, 1993; Kaumann, et al., 1994).

The 5-HT$_5$ receptor.

Two 5-HT$_5$ receptor genes have been cloned in rat and mouse and the recombinant receptors labelled 5-HT$_{5A}$ and 5-HT$_{5B}$ (Plassat et al., 1992; Matthes et al., 1993; Erlander et al., 1993). Despite both receptors having a pharmacological profile similar to that of 5-HT_1 receptors there are a variety of reasons not to place them in this class including the very limited (<37%) sequence homology between 5-HT_1 and 5-HT_5 receptors and the presence of introns in the 5-HT_5 receptor gene but not the 5-HT_1 gene. However, since the transductional mechanisms of these receptors are presently unknown and as such they are not fully characterised, the 5-HT_5 receptor class is presently a provisional classification.

Little is known about the localisation of 5-HT_5 receptors. *In situ* hybridisation studies have indicated the presence of 5-HT_{5A} receptor mRNA in cerebral cortex, hippocampus, habenula, olfactory bulb and the granullar layers of the cerebellum in mice (Plassat et al., 1992). The distribution of the 5-HT_{5B} receptor is far more limited, *in situ* hybridisation studies have revealed signals only in the habenula and the CA1 field of the hippocampus. The functional significance of these receptors is presently unknown.

The 5-HT_6 receptor.

A new 5-HT receptor protein gene has recently been cloned that, that like the 5-HT_4 receptor, positively links to adenylyl cyclase (Monsma et al., 1993; Ruat et al., 1993a). This has been provisionally labelled the 5-HT_6 receptor on the basis of its unique pharmacological profile and its limited sequence homology with other 5-HT receptor genes, including the presence of 2 introns. It must be stressed that the appellation "5-HT_6 receptor" is provisional until the whole-cell function of the receptor has been described and the 5-HT_4 receptor has been cloned. It is possible that the "5-HT_6 receptor" is a member of the 5-HT_4 receptor family, although the known pharmacological differences between these 2 receptors suggests that this is unlikely.

The compound with highest affinity for the receptor is methiothepin. Various ergolines, including, LSD, lisuride and metergoline also have high affinity for this receptor. The most remarkable feature of the pharmacology of the 5-HT_6 receptor is its high affinity for antipsychotic and antidepressant drugs, such as clozapine, chlorprothixene and fluphenazine, which all acted as antagonists (Roth et al., 1994).

Northern blot studies of the distribution of 5-HT_6 receptor mRNA have localised it exclusively in the brain, with highest densities in the striatum, olfactory tubercle, cerebral cortex and hippocampus.

The 5-HT_7 receptor.

A putative 5-HT_7 receptor gene has been cloned from rat, mouse and human central nervous system tissue (Bard et al., 1993; Lovenberg et al., 1993; Plassat, et al., 1993; Ruat, et al, 1993b; Meyerhof et al., 1993; Shen et al., 1993). The definition of the 5-HT_7 receptor is based upon the sequence of its gene, which has limited homology with other cloned 5-HT receptors and functional studies which have demonstrated that the receptor has high affinity for 5-HT and is positively coupled to adenylyl cyclase.

The mRNA for the 5-HT_7 receptor has been localised both in the central nervous system, predominantly in the thalamus and hypothalamus, but also in the medulla, hippocampus and cortex and also peripherally in the gastrointestinal tract (Lovenberg et al., 1993; Ruat, et al., 1993). The 5-HT_7 receptor has high affinity for a variety of both 5-HT_{1A} receptor agonists, such as 8-OH-DPAT and 5-carboxamidotryptamine, and 5-$HT_{2A/C}$ receptor antagonists, such as mesulergine and ritanserin (Lovenberg et al., 1993). At the time that these experiments were performed the β-adrenergic receptor antagonist, pindolol was one of the only ligands known to have high affinity for the 5-HT_{1A} receptor (pK_D 7.71), but low affinity for the 5-HT_7 receptor (pK_D <6.0; Hoyer et al., 1994). It is now known that the novel 5-HT_{1A} antagonist, WAY-100635 at a concentration of 100 nM does not cause significant displacement of specific radioligand binding to the rat 5-HT_7 receptor (Hamblin, unpublished observations). It is possible that reports of 5-HT_{1A}-like receptors located in the hippocampus positively coupled to adenylyl cyclase (Shenker et al., 1987) and sympathoexcitatory 5-HT_{1A} receptors located in the forebrain (Anderson et al., 1992) may prove to be 5-HT_7 receptors.

Summary and Aims of the thesis.

There is evidence to support the view that there is an excitatory 5-HT-containing pathway innervating cardiac vagal motoneurones that involves activation of 5-HT$_{1A}$ receptors. 5-HT$_{1A}$ receptors are located in brain areas containing cardiac vagal motoneurones, the NA and DVMN of cats (Dashwood et al., 1988) and rats (Thor et al., 1991). The NA and DVMN contain 5-HT-like immunoreactive neurones in rats (Steinbusch et al., 1981) and cats (Izzo et al., 1988). Furthermore, boutons with 5-HT-like immunoreactivity make synaptic contact with retrogradely labelled cardiac vagal motoneurones (Izzo et al., 1988) and micro-injection of 5-HT or 5-HT$_{1A}$ agonists into the vicinity of cardiac vagal motoneurones causes a bradycardia in cats (Izzo et al., 1988) and rats (Sporton et al., 1991).

Stimulation of the upper airways of the anaesthetised, atenolol pretreated rabbit with cigarette smoke elicits a reflex vagal bradycardia associated with an apnoea, renal sympathoexcitation and increase in arterial blood pressure. Given the above evidence it would be predicted that 5-HT$_{1A}$ agonists should potentiate the bradycardia. However, it has previously been demonstrated that administration of the 5-HT$_{1A}$ agonist 8-OH-DPAT i.c. inhibits this bradycardia and the 5-HT$_{1A}$ partial agonist buspirone potentiates the bradycardia (Futuro-Neto et al., 1993). In the present study the following possibilities were investigated;

1. A species difference exists between rabbits and rats with respect to the role played by 5-HT$_{1A}$ receptors in the modulation of cardiac vagal motoneurones.

2. That 8-OH-DPAT or buspirone may have had actions in rabbits at receptors other than the 5-HT$_{1A}$ receptor.

3. That the effects of buspirone and 8-OH-DPAT on the reflex bradycardia in rabbits may have been attributable to the changes in respiratory drive caused by these drugs.

4. The role of other 5-HT receptors located in the medulla in the modulation of CVM activity, particularly 5-HT$_{1D}$, 5-HT$_2$, 5-HT$_3$ and 5-HT$_7$ receptors.

Measurement of cardiovascular variables.

The right axillary artery was cannulated (Portex non-sterile polythene tubing, 0.96 mm external diameter, 0.58 mm internal diameter, length 400 mm; heparin saline filled, 25 i.u. ml^{-1}) for the measurement of blood pressure using a pressure transducer (Gould) and the position of the blood pressure transducer was adjusted so that it was at the same height as the heart. Heart rate was derived electronically from the blood pressure signal (RFH Medical Electronics Department). The blood pressure and heart rate were continuously displayed on a chart recorder (Grass Instruments). The output of the blood pressure transducer was also relayed to an analogue to digital converter (ADC) input on the 1401+ computer interface (Cambridge Electronic Design), allowing the signal to be recorded on the hard disk using Spike2 (sampling rate 100 Hz). Blood pressure was subsequently analysed using the Spike2 data analysis programme. In addition, E.C.G. was recorded to allow R-R interval to be measured. E.C.G. was recorded as lead II using silver needle electrodes inserted into the right fore foot, left hind foot and an earth electrode inserted into the muscle of the neck. The signal was amplified (Digitimer NL104; gain 2K) and filtered (Digitimer NL125; frequency band 100-500 Hz). The R waves of the E.C.G. were detected using a spike processor (Digitimer D130) and the signal applied to the digital input of the 1401+ interface (sampled at 1KHz) allowing the R-R interval to be calculated using Spike2 data analysis.

Cannulation of other blood vessels.

The right and left axillary veins were cannulated (Portex non-sterile polythene tube, external diameter 0.96 mm, internal diameter 0.58 mm, volume 100 μl, saline (0.9 % w/v) filled) to allow drugs and an infusion and an infusion respectively to be administered i.v. The infusion solution consisted of 50 ml distilled water and 50 ml gelofusin, containing 0.84% (w/v) sodium bicarbonate and 0.2% (w/v) glucose, infused at 6 ml kg^{-1} $hour^{-1}$ (Watson Marlow 501U infusion pump) this was to prevent non-respiratory acidosis and maintain blood volume. The left axillary artery was cannulated (same tube type as above, length 400 mm, heparin saline filled 25 i.u. ml^{-1}) to allow blood samples to be taken (100 μl) in order to measure arterial blood gases (Ciba-Corning 238 pH/blood gas analyser; Pa_{O_2} 100 - 140 mmHg; Pa_{CO_2} 30 - 40 mmHg; HCO_3 12.0 - 20.0 mMol l^{-1}; pH 7.30 - 7.45). In some experiments the right internal jugular vein was cannulated (same tube type as above, volume 100 µl, filled with phenylbiguanide (1 mg ml^{-1}; dissolved in saline 0.9 % w/v) the cannula was advanced 7 cm so that the tip lay within, or very close to the right atrium.

Insertion of bladder catheter.

A midline incision was made from 20 mm superior to the pubic symphysis to the level of the iliac crest. A 60 mm incision through the linea alba was made to expose the bladder. The bladder was emptied of urine using a 20 ml syringe and a catheter (polythene tubing, external diameter 3.5 mm, internal diameter 2.0 mm) was inserted so that urine could drain freely. The abdominal wall was closed using 3 michel clips (Downs Surgical Ltd; AT1) and the wound was covered with saline moistened cotton wool.

Positioning rabbit in head frame, exposure of atlanto-occipital membrane.

The right and left zygomatic arches of the maxillary bone were then exposed and a small indentation was drilled into both zygomaticofacial foramina so that the rabbit, lying on its right side, could be placed in a head holder (made by RFH medical engineering). A midline incision was made in the skin over the cervical vertebrae, extending rostrally as far as the lambdoid suture of the skull, the underlying muscle was removed by blunt dissection and cautery and the atlanto-occipital membrane was exposed. The membrane was pierced with a needle made from 28 gauge hollow stainless steel tubing and connected to a 100 µl Hamilton syringe by 320 mm of polythene tubing (Portex non-sterile, external diameter 0.61 mm, internal diameter 0.28 mm). This allowed the administration of drugs intracisternally (i.c.) dissolved in a volume of 20 µl and given over 20 s.

Phrenic nerve recording.

The left phrenic nerve was exposed low in the neck using a lateral approach by cutting and reflecting the levator scapulae muscle, previously exposed during the dissection to reach the atlanto-occipital membrane. The phrenic nerve runs down the anterior surface of the scalenus anterior muscle, behind the prevertebral layer of deep fascia. The fascia was removed by blunt dissection, the nerve was cleaned of excess connective tissue, cut distally and then placed on a bipolar silver hook electrode. The nerve and electrode were then covered with approximately 2 ml of President light body dental polyvinylsiloxane (Coltene; Art. no. 4671) this solidified to hold the nerve in place on the electrode and prevent it either drying out or signal attenuation by saline short circuiting the electrode. Phrenic nerve activity was amplified (Digitimer NL104), filtered (Digitimer NL125; frequency band 0.2-2 KHz) and recorded onto hard disk via a 1401+ interface using Spike2 data collection (sampling rate 1 KHz).

Renal nerve recording.

The left kidney was exposed using a retroperitoneal approach. A 10 cm skin incision was made above the position where the kidney could be palpated. An incision was made through the external oblique, internal oblique and transversus abdominis muscles using cautery and the edges of the wound were retracted to expose the kidney and renal vessels lying on the peritoneum. The kidney was deflected laterally to expose the renal vessels, along which the renal nerve travels. The nerve was dissected free of the surrounding connective tissue, taking great care to preserve the integrity of the peritoneum. Once free it was placed on a bipolar silver hook electrode. The renal nerve activity (RNA) was amplified (Digitimer NL104), filtered (Digitimer NL125; frequency band 100-500 Hz) and integrated using an E.M.G. integrator (Digitimer NL703) to rectify the signal and smooth it with a 20 ms time constant. RNA was recorded onto hard disk using a 1401+ interface and Spike2 with a 1 KHz sampling rate. The output from the E.M.G. integrator was sampled at 100 Hz and recorded onto hard disk using a 1401+ interface and Spike2.

Experimental protocol, reflexes evoked in rabbits.

Reflex responses to smoke; spontaneously breathing, normoxic rabbits.

Experiments were performed in rabbits spontaneously breathing room air. The preparation was allowed to stabilise for at least 30 min before the protocol was started. Experiments were performed only in rabbits with a stable mean resting R-R interval and stable smoke responses with an R-R interval increase of greater than 25 ms and an apnoea duration of greater than 5 s. Following the stabilisation period smoke challenges were performed at 10 min intervals (see figure 2.1) using a volume of smoke (15-45 ml) that was chosen to produce a submaximal reflex bradycardia. Smoke was produced from commercially available nicotine free herbal cigarettes (Honeyrose Products Ltd). It was collected in a 50 ml glass syringe and then blown at a constant rate through the upper airways over approximately 5 s. In the intervening periods, warmed and humidified air was passed through the upper airways at the rate of 10 ml s^{-1}. This was done to prevent smoke remaining in the upper airways and damaging them during the experiment and to control for the stimulation of flow receptors during the smoke challenges. The smoke challenges were repeated until 3 consecutive heart rate responses of similar magnitude were produced. 5 min after the third challenge, 1 mg kg^{-1} of atenolol, a selective β_1-adrenoceptor antagonist was administered i.v. 5 minutes later the smoke challenges were resumed and repeated until 3 consecutive responses of similar magnitude were produced. This usually took 4 challenges, if more than 6 were required the experiment was abandoned. At least 40 min after the dose of atenolol the test drug was administered either i.c. or i.v. in a volume of 20 μl given over 20 s. 5 minutes later the smoke challenges were resumed at 10 min intervals.

In some experiments a second test drug was given i.c. 20 min after initial pretreatment with a drug either i.v. or i.c. In all experiments, smoke challenges were repeated until 35 min after the final dose of test drug.

Figure 2.1

Timing of experimental protocols in rabbits and rats.

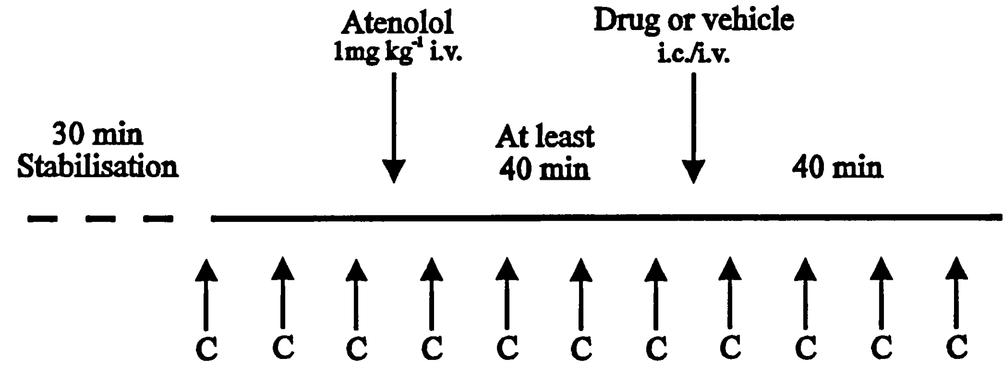
1. Administration of a drug i.v. or i.c. to an atenolol pretreated animal.

2. Administration of a drug i.v. or i.c. following pretreatment with another drug i.v. or i.c. to an atenolol pretreated animal.

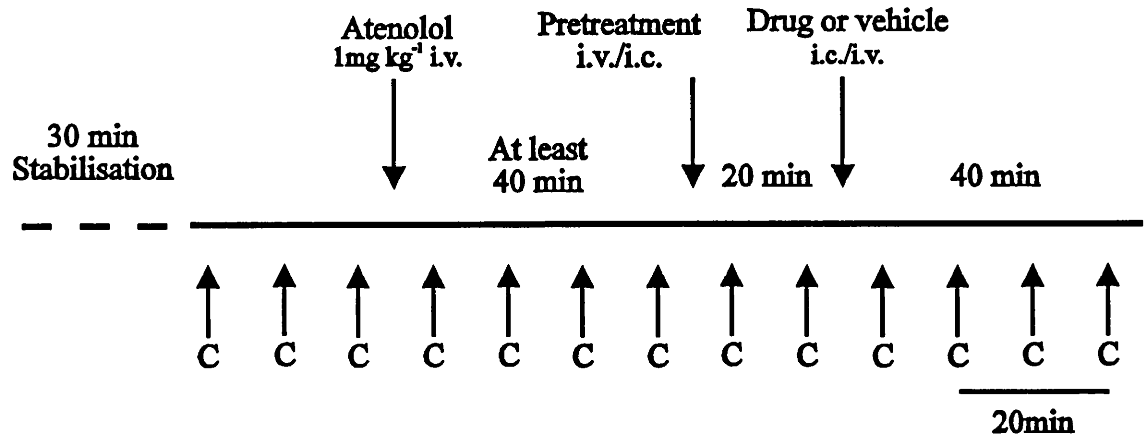
C = Smoke or phenylbiguanide challenge.

Figure 2.1 Rabbit and rat experimental protocols; 1. Drug alone; 2. Pretreatment then drug.

1.



2.



C = Smoke or PBG challenge

Reflex responses to smoke; ventilated, normoxic rabbits.

Experiments were performed in artificially ventilated rabbits. The rate of ventilation was 60 cycles min^{-1} and the volume was 10 - 15 ml, adjusted to maintain blood gases within the limits stated above. If necessary the inspired room air could be enriched with up to 100 % O_2. The rest of the protocol was the same as above.

Reflex responses to smoke; spontaneously breathing, hyperoxic rabbits.

Experiments were performed on hyperoxic rabbits. An oxygen mask was placed over the lower tracheostomy tube so that the rabbit inspired 100 % O_2, to maintain Pa_{O_2} between 180 - 200 mmHg. At this level of hyperoxia the Pa_{O_2} never fell below 120 mmHg during the smoke response apnoea. The rest of the protocol was the same as above.

Reflex responses to phenylbiguanide; spontaneously breathing, normoxic rabbits.

The same protocol was used in experiments in rabbits stimulated by administration of phenylbiguanide (PBG) into the right atrium, with the following modifications. A catheter (Portex non-sterile polythene tubing, external diameter 0.96 mm, internal diameter 0.58 mm, volume 100 μl, filled with a solution containing PBG (1 mg ml^{-1}) dissolved in saline 0.9 % w/v) was inserted into the right jugular vein of the rabbit and advanced 7 cm so that the tip of the cannula was located within, or very close to the right atrium. A dose of PBG (10 - 25 μg kg^{-1}) was then chosen to produce a submaximal bradycardia of between 25 and 100 beats min^{-1}. The challenges were repeated and drugs were given at the same intervals as were used in the smoke response experiments.

Measurement of the circulation time in rabbits.

The circulation time between the right atrium and the carotid body was estimated by measuring the time between injecting a bolus of saline and a reduction in the temperature of a thermocouple (copper/copper-nickel welded tip; polytetra-fluoroethylene insulated; 0.6 mm diameter; RS type K) located in the left external carotid artery. Using the approach used to perform the tracheostomies, the thermocouple was inserted into the left lingual artery and advanced until the tip could be seen in the external carotid artery. A 0.5 ml bolus of saline (21°C) was injected into a cannula inserted 7 cm into the jugular vein and the marker channel was triggered using the foot pedal. The bolus of saline had no effect on heart rate or blood pressure. The thermocouple was connected to a digital thermometer (Digitron), the output of which was amplified using a neurolog amplifier (NL104) and stored on hard disk using Spike2 (100 Hz sampling rate). An estimation of the transit time was obtained by subtracting the time when the marker channel was triggered from the time at which the temperature of the thermocouple started to change.

Preparation of the rats.

Anaesthesia.

Male Sprague-Dawley rats (180 - 320 g) were used. Anaesthesia was induced using halothane (4 % in 100 % O_2 at 1 l min^{-1}) and maintained using between 0.5% and 2 % halothane with the same O_2 flow rate while the right femoral vein was cannulated with polythene tubing (Portex non-sterile, external diameter 0.96 mm, internal diameter 0.58 mm, volume 100 μl, saline filled). The halothane was then removed and anaesthesia was maintained using 1.5 g kg^{-1} urethane i.v. (25 % w/v, dissolved in distilled water). This was supplemented as necessary with 0.15 g kg^{-1} urethane i.v. Anaesthetic level was assessed using stability of blood pressure and heart rate, regularity and rate of phrenic nerve activity and absence of limb withdrawal in response to paw pinch. The body temperature was measured with a rectal temperature probe and maintained between 37-38 °C using a homeothermic electric blanket system (Harvard).

Tracheal cannulation.

A midline incision was made in the neck between the level of the suprasternal notch and the hyoid bone. The underlying muscles were divided by blunt dissection and a tracheal tube was inserted low in the trachea to allow the rat to spontaneously breath room air. The tracheal tube was made of polythene, length 40 mm, external diameter 2 mm, internal diameter 1.75 mm. The mouth was blocked using a small piece of saline moistened cotton wool and a second tracheal tube (polythene; 2 mm external diameter, 1.75 mm internal diameter, 150 mm long) was inserted 3 mm rostrally, from a point 5 mm below the cricoid cartilage of the larynx to allow smoke to be passed through the larynx and upper airways and out through the nostrils. A bolus of smoke was then administered through the upper airway tracheal tube to check that smoke exited only through the nostrils.

Measurement of cardiovascular variables.

The right femoral artery was cannulated (Portex non-sterile polythene tubing, 0.96 mm external diameter, 0.58 mm internal diameter, length 400 mm; heparin saline filled, 25 i.u. ml^{-1}) for the measurement of blood pressure using a pressure transducer (Gould) and heart rate which was derived electronically from this signal (RFH Medical Electronics Department). Both outputs were continuously displayed on a chart recorder (Grass Instruments). The output of the blood pressure transducer was also relayed to an analogue to digital converter (ADC) input on the 1401+ interface, allowing the signal to be recorded on the hard disk using Spike2 (sampling rate 100 Hz). Blood pressure was subsequently analysed using the Spike2 data analysis programme. In addition, E.C.G. was recorded as lead II using silver needle electrodes inserted into the right fore foot, left hind foot and an earth electrode inserted into the muscle of the neck to allow R-R interval to be calculated. The signal was amplified (Digitimer NL104; gain 2K) and filtered (Digitimer NL125; frequency band 100-500 Hz). The R waves of the E.C.G. were detected using a spike processor (Digitimer D130) which sent a signal to the digital input of the 1401+ interface allowing E.C.G. to be recorded using Spike2, (sampled at 1 KHz) and the R-R interval to be calculated using Spike2 data analysis.

Cannulation of other blood vessels.

The right axillary vein was cannulated to allow drugs to be administered i.v (Portex non-sterile polythene tube, external diameter 0.96 mm, internal diameter 0.58 mm, volume 100 μl, saline filled (0.9 % w/v). In some experiments the internal jugular vein was cannulated (same tube type as above, volume 100 μl, filled with phenylbiguanide

(0.1 mg ml^{-1}; dissolved in saline 0.9 % w/v). The tube was advanced 3 cm so that the tip lay in or very close to the right atrium.

Positioning the rat in a headframe and exposure of the atlanto-occipital membrane.

The rat and electric blanket were placed on a height adjustable platform to facilitate subsequent surgery. Ear bars were placed into the external auditory meatus, the upper incisors were placed over a tooth bar and the rat was positioned prone with the ear bars raised 55 mm above the level of the platform. The position of the blood pressure transducer was adjusted until it was at the same height as the heart. A midline incision was made in the skin over the cervical vertebrae, extending rostrally as far as the lambdoid suture of the skull, the underlying muscle was removed by blunt dissection and cautery and the atlanto-occipital membrane was exposed. The membrane was pierced with a needle made from 28 gauge hollow stainless steel tubing and connected to a 100μl Hamilton syringe by 320 mm of polythene tubing (Portex non-sterile, external diameter 0.61 mm, internal diameter 0.28 mm). This allowed the administration of drugs intracisternally (i.c.) dissolved in a volume of 20 μl and given over 20 s.

Whole nerve recording of the phrenic nerve.

The head frame was tilted 45° to the right. The trapezius and latissimus dorsi muscles had already been exposed by the dissection to expose the atlanto-occipital membrane. These muscles and the underlying levator scapulae, rhomboid major and rhomboid minor muscles were cut to allow the scapula to be reflected ventrally. This exposed the brachial plexus, which was dissected free of the surrounding connective tissue and ligated. The brachial plexus was then cut distal to the ligature, taking care to avoid the axillary artery and vein. The proximal portion of the brachial plexus was then reflected dorsally using the ligature. This exposed the phrenic nerve, which passes vertically downwards along the ventral aspect of the scalenus anterior muscle, behind the prevertebral layer of deep fascia. The fascia was removed by blunt dissection, the nerve was cleaned of excess connective tissue, cut distally and the central end placed on a bipolar silver hook electrode. The nerve and electrode were then covered with approximately 2 ml of President light body dental polyvinylsiloxane (Coltene; Art. no. 4671) this solidified to hold the nerve in place on the electrode and prevent it either drying out or signal attenuation by saline short circuiting the electrode. Phrenic nerve activity was amplified (Digitimer NL104), filtered (Digitimer NL125; frequency band 0.2 - 2 KHz) and recorded onto hard disk via the 1401+ interface using Spike2 data collection (sampling rate 1 KHz).

Experimental protocol, reflexes evoked in rats.

Reflex responses to smoke.

Experiments were performed in rats spontaneously breathing room air. The preparation was allowed to stabilise for 30 min before the protocol was started. Experiments were performed only in rats with a stable mean resting R-R interval and when a stable reflex could be obtained with an R-R interval increase of greater than 15 ms and an apnoea of greater than 3 s duration. Following the stabilisation period smoke challenges were performed at 10 min intervals (see figure 2.1) using a volume of smoke (5-30 ml) that was chosen to produce a submaximal reflex bradycardia. The smoke was produced using commercially available nicotine free herbal cigarettes (Honeyrose Products Ltd). It was collected in a 50 ml glass syringe and then blown smoothly through the upper airways over approximately 3s. The smoke challenges were repeated at 10 min intervals until 3 consecutive responses of equal magnitude were produced. 5 min after the third challenge, 1 mg kg^{-1} of atenolol, a selective β_1 adrenoceptor antagonist was administered i.v. 5 minutes later the smoke challenges were resumed at 10 min intervals and repeated until 3 consecutive responses of equal magnitude were produced. At least 40 min after the dose of atenolol the test drug was administered either i.c. or i.v. in a volume of 20 μl given over 20 s. 5 minutes later the smoke challenges were resumed at 10 min intervals. In some experiments a second test drug was given i.c. 20 min after the first. In all experiments, smoke challenges were repeated until 35 min after the final dose of test drug.

Reflex responses to phenylbiguanide.

The same protocol was used in experiments in rats stimulated by administration of phenylbiguanide (PBG) into the right atrium, with the following modifications. A catheter (Portex non-sterile polythene tubing, external diameter 0.96 mm, internal diameter 0.58 mm, volume 100 μl, filled with a solution of PBG (0.1 mg ml^{-1}) dissolved in saline 0.9 % w/v) was inserted into the right jugular vein of the rabbit and advanced 3 cm so that the tip of the cannula was located within, or very close to the right atrium. A dose of PBG (10 - 25 μg kg^{-1}) was then chosen to produce a submaximal bradycardia of between 25 and 100 beats min^{-1}. The challenges were repeated and drugs were given at the same intervals as were used in the smoke response experiments.

Analysis of results.

R-R interval changes.

R-R interval was calculated from the E.C.G. signal or pulse interval was derived from the systolic blood pressure signal using Spike2 data analysis scripts written for this work. When a stimulus was applied to the animal, a marker channel was triggered using a foot pedal. This mark was later located using a Spike2 script and the resting R-R interval was measured 2s before this point. If the resting R-R interval was constant, as it usually was in atenolol pretreated animals, the instantaneous R-R interval at this point was measured. If there was some variation in the resting R-R interval, for instance due to sinus arrhythmia, a visual average was measured by placing the horizontal cursor in the centre of the R-R interval range over a 30 s period. Experiments were not performed on animals with an unstable mean resting R-R interval. In rabbits, both the maximal instantaneous R-R interval and the instantaneous R-R interval 3s after the mark were then measured. The resting R-R interval was then subtracted from these R-R interval values to give the changes in R-R interval. The 3s R-R interval change was measured since at this point the reflex bradycardia was due to stimulation of nasopharyngeal receptors in the absence of chemoreceptor activity and this analysis also allows the effect of variations in apnoea duration on R-R interval change to be excluded. After 3s the carotid bodies of the rabbit will still be receiving blood oxygenated before any reflex apnoea was triggered. Similar analysis was performed on the data obtained from rat experiments, but the resting R-R interval was subtracted only from the longest R-R interval, since in experiments that resulted in shortening of the apnoea in rabbits tended to abolish the apnoea in the rats. For the Spike2 script written to analyse these data see appendix 5.1. The effects of the drugs on resting R-R interval were calculated by comparing the resting R-R interval 5 min before administration of drug with that 5 min after and thereafter at 10 min intervals until 35 min had elapsed.

Changes in blood pressure.

The stimulus mark was located and the systolic and diastolic blood pressures were measured 2s before this point.. The peak change of systolic pressure and the diastolic pressure at this point were also measured. Mean arterial pressure (MAP) was calculated as diastolic pressure + ((systolic pressure - diastolic pressure)/3) and changes in the MAP (response MAP - baseline MAP) were calculated for each response. Similar analyses were performed on the data obtained from rat experiments. For the Spike2 script written to analyse these data see appendix 5.2.

Analysis of renal nerve activity.

Renal nerve activity (RNA) was rectified and integrated with a 20 ms time constant and integrated using a Digitimer EMG integrator (NL703). A 20 ms time constant was chosen to allow the integration channel to be sampled at the relatively slow rate of 100 Hz. This was necessary because the Spike2 programme used to analyse the data can only hold 16000 data points at a time. The mean level over 30s of integrated RNA was then measured using a Spike2 script (see Appendix 5.3). The mean level of the integrated RNA was measured for 30s before and 30s after the smoke challenge. The RNA levels before and after the smoke challenge were analysed separately. The mean level of RNA 5 min before drug treatment was deemed to be 100 % and changes thereafter are expressed as a percentage of this level. In addition the RNA was integrated (RFH medical electronics department solid state integrator) with a 5s time constant to produce the RNAint traces used to illustrate the figures in this thesis. 5s was chosen as the time constant because it produced the clearest figures.

Measurement of phrenic nerve activity.

The raw phrenic nerve activity (PNA) channel was displayed using the Spike2 data analysis programme and the beginning and end of the apnoea (cessation of rhythmical bursting) were labelled using vertical cursors. The duration of the apnoea was then calculated using a Spike2 script, a copy of which is supplied in appendix 1.4. In addition the rate of phrenic bursts was counted over 30 s to obtain the number of phrenic bursts min^{-1}.

Statistical analysis of data.

All statistical analyses were performed by 2 way ANOVA, subsequently analysed using the least significant difference test (Sokal & Rohlf, 1967), giving time matched comparison of drug-induced changes with those of vehicle. The change was calculated by subtracting the absolute value at a given time after treatment from the absolute value 5 min before treatment. The absolute values and the changes of these values were compared with the values obtained for the vehicle after the same time period. All values are expressed as the mean ± s.e. mean. Differences in the mean were taken as significant when $p < 0.05$.

Drugs and solutions.

The following drugs were dissolved in 0.9 % w/v saline.

Atenolol (Sigma Chemical Co., Poole, U.K.). β_1-adrenoceptor antagonist, does not penetrate blood brain barrier or bind to 5-HT_{1A} receptors.

Atropine methylnitrate (Sigma Chemical Co., Poole, U.K.). Muscarinic acetylcholine receptor antagonist, penetrates blood brain barrier less than atropine sulphate.

Buspirone (8-(4-(4-(2-pyrimidinyl)-1-piperazinyl)butyl)-8-azaspirol(4.5)decane-7,9-dione HCl; Research Biochemicals Inc., Semat, St. Albans, U.K.). 5-HT_{1A} receptor partial agonist (pK_D 7.58), similar affinity for D_2 dopamine receptors where it is an antagonist.

nn-DP-5-CT (n,n-di-n-propyl-5-carboxamidotryptamine maleate; Research Biochemicals Inc., Semat, St. Albans, U.K.). 5-HT_{1A} receptor agonist (pK_D 9.54) also has 5-HT_{1D} receptor agonist action (pK_D 7.24).

Granisetron (endo-n-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)-1-methyl-1H-indazole-3-carboxamide HCl; a gift from SmithKline Beecham Pharmaceuticals, Worthing, U.K.) 5-HT_3 receptor antagonist (pK_D 8.9).
(+)8-OH-DPAT ((+)8-hydroxy-2-(di-N-propylamino)tetralin HBr; Research Biochemicals Inc., Semat, St. Albans, U.K.). 5-HT_{1A} receptor agonist (pK_D 8.7) also has 5-HT_{1D} receptor agonist action (pK_D 6.9).
Sumatriptan (3-(2-(dimethylamino)ethyl)-N-methyl-1H-indole-5-methanesulphonamide succinate; a gift from Wellcome Laboratories, Beckenham, U.K.). 5-HT_{1D} receptor agonist (pK_D 7.5) also has 5-HT_{1A} receptor agonist action (pK_D 6.1).
WAY-100635 (n-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-n-(2-pyrinidinyl)cyclohexanecarboxamide 3HCl; a gift from Wyeth Research Ltd., Maidenhead, U.K.). 5-HT_{1A} receptor antagonist (IC_{50} 2 nM), has slight affinity for α_1-adrenoceptors (IC_{50} 230 nM).
WAY-100802 ((R)-2,3,4,5,6,7-hexahydro-1-(4-(1-(4-(2-methoxyphenyl)piperazinyl))-2-phenyl)butanoyl-1H-azepine HCl; a gift from Wyeth Research Ltd., Maidenhead, U.K.). Novel 5-HT_{1A} receptor antagonist (IC_{50} 1.4 nM), has slight affinity for α_1-adrenoceptors (IC_{50} 682 nM).

The following drugs were dissolved in 0.01N hydrochloric acid and then the solutions were diluted with 0.9 % w/v saline.
(-)Pindolol (1-(1H-indol-4-yloxy)-3-[(1-methylethyl)amino]-2-propanol; Research Biochemicals Inc., Semat, St. Albans, U.K.). β_1-adrenoceptor antagonist with 5-HT_{1A} receptor antagonist action (pK_D 7.8), similar affinity for 5-HT_{1B} receptors.
Sulpiride (5-(aminosulphonyl)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide; Research Biochemicals Inc., Semat, St. Albans, U.K.). D_2 dopamine receptor antagonist. Control for D_2 dopamine receptor antagonist action of buspirone.

The following drugs were dissolved in distilled water.
GR-127935 (n-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide; a gift from Glaxo Group Research Ltd., Ware, U.K.). 5-HT_{1D} receptor antagonist (pK_D 9.3) also has 5-HT_{1A} receptor antagonist action (pK_D 6.9).
Mesulergine (n-[(8α)-1,6-dimethylergolin-8-yl]-n,n-dimethylsulphamide HCl; Research Biochemicals Inc., Semat, St. Albans, U.K.). $5\text{-HT}_{2A/C}$ receptor antagonist (pK_D 8.8/8.4) also has 5-HT_7 receptor antagonist action.
Urethane (Sigma Chemicals Ltd., Poole, U.K.). Maintenance general anaesthetic (i.v.).

Halothane (I.C.I. Pharmaceuticals Ltd., Macclesfield, U.K.). Inhalation general anaesthetic used for induction of rats.
Sodium pentobarbitone (Sigma Chemicals Ltd., Poole, U.K.). General anaesthetic used for euthanasia.

Results

Reflex responses to upper airway stimulation of the rabbit with smoke.

Passing a bolus of nicotine-free herbal cigarette smoke (15 - 45 ml) through the upper airways of spontaneously breathing, anaesthetised rabbits (n = 84) triggers a group of responses which have been termed the "diving response". There was an expiratory apnoea of 20 ± 4 s. This apnoea was accompanied by a reflex increase in the R-R interval of 116 ± 33 ms from a resting R-R interval of 260 ± 10 ms. The increase in R-R interval was rapidly returned to near baseline on resumption of regular inspiratory activity, although R-R interval could take up to 5 min to return fully to baseline. There was also an increase in mean arterial pressure of 26 ± 3 mmHg, from a resting value of 57 ± 5 mmHg. This increase in mean arterial pressure was probably caused by an increase in total peripheral resistance. The peripheral vasoconstriction was mediated at least in part by increased firing of sympathetic nerves, in these experiments renal nerve activity was recorded and increased by 211 ± 27 % over the period of the response. Again mean arterial blood pressure and renal nerve activity could take up to 5 min to return to baseline. A smoke response is illustrated in Figure 3.1.

Experiments on spontaneously breathing rabbits, stimulated with smoke.

These results are summarised in Table 3.13.

Effects of vehicles.

Resting values.

Intracisternal injection of saline (20 μl; n=5; Figure 3.4 and Table 3.1), acidified saline (20 μl; n=5; pH = 2.0; Figure 3.11 and Table 5.14 in Appendix) and distilled water (20 μl; n=4; Table 5.38 in Appendix), in separate experiments, had little effect on the resting R-R interval, mean arterial blood pressure, renal nerve activity and the rate of phrenic bursts.

Reflex responses to smoke.

Neither saline (Figure 3.5 and Table 3.2), acidified saline (Figure 3.12 and Table 5.35 in Appendix) nor distilled water (Figure 3.17 and Table 3.14) caused change to the smoke evoked increase in R-R interval, mean arterial blood pressure, apnoea duration or renal nerve activity.

Effects of 5-HT_{1A} receptor ligands.

Buspirone (i.c.).

Resting values.

Intracisternal (i.c.) administration of buspirone (200 μg kg^{-1}; n=5; Figure 3.4 and Table 3.1) caused significant ($p<0.05$) changes in all resting variables when compared to i.c. saline (20 μl; n=5). The resting R-R interval increased maximally by 23 ± 8 ms 5 min after administration of buspirone and this was associated with a significant increase in the number of phrenic bursts by 25 ± 13 bursts min^{-1}. A non-significant decrease in renal nerve activity of -37 ± 21 % and a significant decrease in mean arterial blood pressure of -12 ± 4 mmHg. The decline in renal nerve activity continued over the duration of the experiment, reaching significance 25 min after administration of buspirone by -56 ± 17%. At this time point the increase in phrenic nerve activity burst rate was also maximum (50 ± 11 bursts min^{-1}).

Reflex responses to smoke.

5 min after administration of buspirone the peak smoke evoked reflex increase in the R-R interval was near maximally potentiated by 162 ± 82 ms (Figure 3.5 and Table 3.2), this was significant when compared with saline (i.c.). This was associated with a significant inhibition of the pressor response of -12 ± 4 mmHg. Furthermore, at 5 min the reflex increase in R-R interval was significantly potentiated at 3s after stimulation with smoke, with an increase of 55 ± 19 ms. However, buspirone had no significant effect on the duration of the apnoea and the increase in renal nerve activity evoked by the smoke stimulus to the upper airway over the period of the experiment. A trace of one of these experiments is shown in Figure 3.1.

(+)8-OH-DPAT (i.c.).

Resting values.

Administration of (+)8-OH-DPAT (i.c.; 25 μg kg^{-1}; n=5; Figure 3.6 and Table 3.3) caused significant changes to resting R-R interval, phrenic burst rate and renal nerve activity. The resting R-R interval was increased significantly after 25 min, by 22 ± 5 ms. This was associated with a significant increase in phrenic burst rate of 92 ± 24 bursts min^{-1} and a significant reduction in renal nerve activity of -28 ± 11 %, but no change in MAP. However, both the phrenic burst rate and renal nerve activity were significantly altered 5 min after administration of (+)8-OH-DPAT in the absence of an increase in R-R interval.

Reflex responses to smoke.

5 min after administration of (+)8-OH-DPAT the peak smoke evoked reflex increase in the R-R interval was near maximally inhibited by -98 ± 23 ms (Figure 3.7 and Table 3.4), this was significant when compared with saline (i.c.). There was no significant change in the R-R interval at 3s. This inhibition of the bradycardia at 5 min was associated with significant reductions in the duration of the apnoea by -11 ± 3 s and the smoke evoked increase in renal nerve activity by -51 ± 10 %. Only after 15 min was the increase in MAP significantly reduced (-10 ± 2 mmHg). The (+)8-OH-DPAT mediated inhibition of the R-R interval increase was not significantly changed at 3 s after smoke stimulation. A trace of one of these experiments is shown in Figure 3.2.

n,n-DP-5-CT (i.c.).

Resting values.

Administration of nn-DP-5-CT (i.c.; 50 μg kg^{-1}; n=5; Figure 3.6 and Table 3.3) caused significant changes in resting R-R interval, phrenic burst rate and renal nerve activity. The resting R-R interval was significantly increased after 25 minutes by 20 ± 9 ms. This was associated with a significant increase in the phrenic burst rate of 51 ± 19 bursts min^{-1} and a significant reduction of resting renal nerve activity of -43 ± 12 %.

Reflex responses to smoke.

There was no significant change in the peak smoke evoked reflex increase in R-R interval or the duration of the apnoea following n,n-DP-5-CT (Figure 3.7 and Table 3.4). The increase in mean arterial blood pressure was significantly inhibited after 5 minutes by -8 ± 2 mmHg and the reflex increase in renal nerve activity was significantly reduced after 25 minutes by -33 ± 11 %.

(-)Pindolol (i.c.).

Resting values.

Administration of (-)pindolol (i.c.; 100 μg kg^{-1}; n=5; Figure 3.11 and Table 5.16 in Appendix 5.5) caused a significant increase in baseline R-R interval, after 5 min only, of 12 ± 4 ms. This was not associated with significant changes in any of the other resting variables, although they were all reduced. The reductions in phrenic burst rate and resting renal nerve activity were both significant after 15 minutes, when they were reduced by -13 ± 9 breaths min^{-1} and -33 ± 17 % respectively.

Reflex responses to smoke.

15 min after administration of (-)pindolol the peak smoke evoked increase in the R-R interval was significantly inhibited by -207 ± 60 ms (Figure 3.12 and Table 5.37 in Appendix 5.5). This was associated with a significant reduction in the smoke evoked increase in mean arterial blood pressure of -16 ± 5 mmHg and a significant inhibition of the reflex increase in renal nerve activity of -41 ± 12 %. However, only after 25 min was the apnoea duration significantly potentiated by 24 ± 7 s.

WAY-100635 (i.c.).

Resting values.

Administration of WAY-100635 (i.c.; 100 μg kg^{-1}; n=5; Table 5.5 in Appendix 5.5) caused no significant changes to any of the baseline variables.

Reflex responses to smoke.

WAY-100635 caused a significant inhibition of the peak reflex increase in R-R interval after 5 min, by -68 ± 27 ms (Figure 3.8 and Table 3.5). This was associated with a significant inhibition of the increase in mean arterial blood pressure (-9 ± 5 mmHg). There were no significant changes to the apnoea duration or the increase in renal nerve activity. A trace of one of these experiments is shown in Figure 3.3.

WAY-100635 (i.v.).

Resting values and Reflex responses to smoke.

Intravenous (i.v.) administration of WAY-100635 (100 μg kg^{-1}; n=5) caused no significant changes to either the resting values (Table 5.6 in Appendix 5.5) or the reflex responses to smoke (Table 5.27 in Appendix 5.5) over a period of 20 min.

Pretreatment with WAY-100635 (i.v.) before buspirone (i.c.).

Resting values.

Pretreatment with WAY-100635 (i.v.; 100 μg kg^{-1}) 20 minutes prior to administration of buspirone (i.c.; 200 μg kg^{-1}; n=5; Figure 3.9 and Table 3.6) did not alter the resting variables (see above), but significantly inhibited the effect of buspirone on these variables when compared to buspirone alone (i.c.; 200 μg kg^{-1}; n=5). The buspirone mediated increase in resting R-R interval was significantly inhibited, reduced from 23 ± 8 ms to 6 ± 3 ms at 5 minutes. This was associated with a significant inhibition of the buspirone mediated increase in phrenic burst rate from 25 ± 13 bursts min^{-1} to -3 ± 3 bursts min^{-1} and a significant inhibition of the buspirone mediated reduction in renal nerve activity from -37 ± 21 % to 13 ± 4 %. Buspirone in the presence of WAY-100635 still caused a fall in resting mean arterial blood pressure of -7 ± 4 mmHg.

Reflex responses to smoke.

There was a significant difference between the effect of buspirone on the smoke evoked increase in R-R interval in untreated and WAY-100635 pretreated rabbits (Figure 3.10 and Table 3.7). The potentiation of the reflex R-R interval increase of 162 ± 82 ms after

5 min by buspirone alone was reduced to an increase of 21 ± 19 ms by pretreatment with WAY-100635. The potentiation of the reflex R-R interval increase at 3 s after the smoke challenge was also significantly inhibited, from an increase of 55 ± 19 ms 5 min after buspirone alone, to a change of 0 ± 1 ms 5 min after buspirone in the WAY-100635 pretreated animals. Pretreatment with WAY-100635 also abolished the buspirone mediated attenuation of the pressor response (-5 ± 1 mmHg untreated, 4 ± 2 mmHg pretreated). The buspirone mediated change in the MAP increase was only 4 ± 2 mmHg after WAY-100635, compared with -5 ± 1 mmHg after buspirone alone. Pretreatment with WAY-100635 did not significantly attenuate the effect of buspirone in reducing apnoea duration and the increase in renal nerve activity evoked by the smoke challenge.

Pretreatment with WAY-100635 (i.v.) before (+)8-OH-DPAT (i.c.).

Resting values.

Pretreatment with WAY-100635 (i.v.; 100 μg kg^{-1}; n=4; Figure 3.13, Tables 3.3 and 3.8) attenuated the effects of (+)8-OH-DPAT (i.c.; 25 μg kg^{-1}) alone on resting variables. The (+)8-OH-DPAT mediated increase in resting R-R interval was significantly inhibited, reduced from 17 ± 5 ms to 0 ± 1 ms at 15 minutes. This was associated with a significant inhibition of the (+)8-OH-DPAT mediated increase in phrenic burst rate from 91 ± 24 bursts min^{-1} to 38 ± 11 bursts min^{-1}. The 8-OH-DPAT evoked inhibition of renal nerve activity was also significantly attenuated, from -37 ± 12% to -8 ± 7 % when the rabbit was pretreated with WAY-100635. In the presence of WAY-100635, 8-OH-DPAT still had no significant effect on resting mean arterial pressure.

Reflex responses to smoke.

There was no significant difference between the effect of (+)8-OH-DPAT on the smoke evoked increase in R-R interval in untreated and WAY-100635 pretreated rabbits (Figure 3.14, Table 3.4 and 3.9). However, the (+)8-OH-DPAT mediated inhibition of the apnoea duration of -13 ± 2 s after 15 min was significantly reduced to -5 ± 1 s. The (+)8-OH-DPAT mediated inhibition of the smoke evoked increase in renal nerve activity was also significantly reduced by WAY-100635 pretreatment, from -41 ± 9 % to 0 ± 3%

after 15 min. WAY-100635 pretreatment did not significantly change the inhibition of the smoke evoked rise in mean arterial blood pressure produced by (+)8-OH-DPAT alone.

Figure 3.1

Trace shows the response to smoke delivered to the upper airways, in an atenolol (1 mg kg^{-1}; i.v.) pretreated rabbit, 5 minutes before and 15 minutes after administration of 200 $\mu g\ kg^{-1}$ buspirone i.c.

From the top, the traces illustrate phrenic nerve activity (PNA), renal nerve activity (RNA), renal nerve activity integrated with a 5 s time constant (RNA(int)), heart rate (HR) and blood pressure (BP). Each trace is 120 s in duration.

A bolus of smoke was passed through the upper airways at the point marked by an arrow and the letter S.

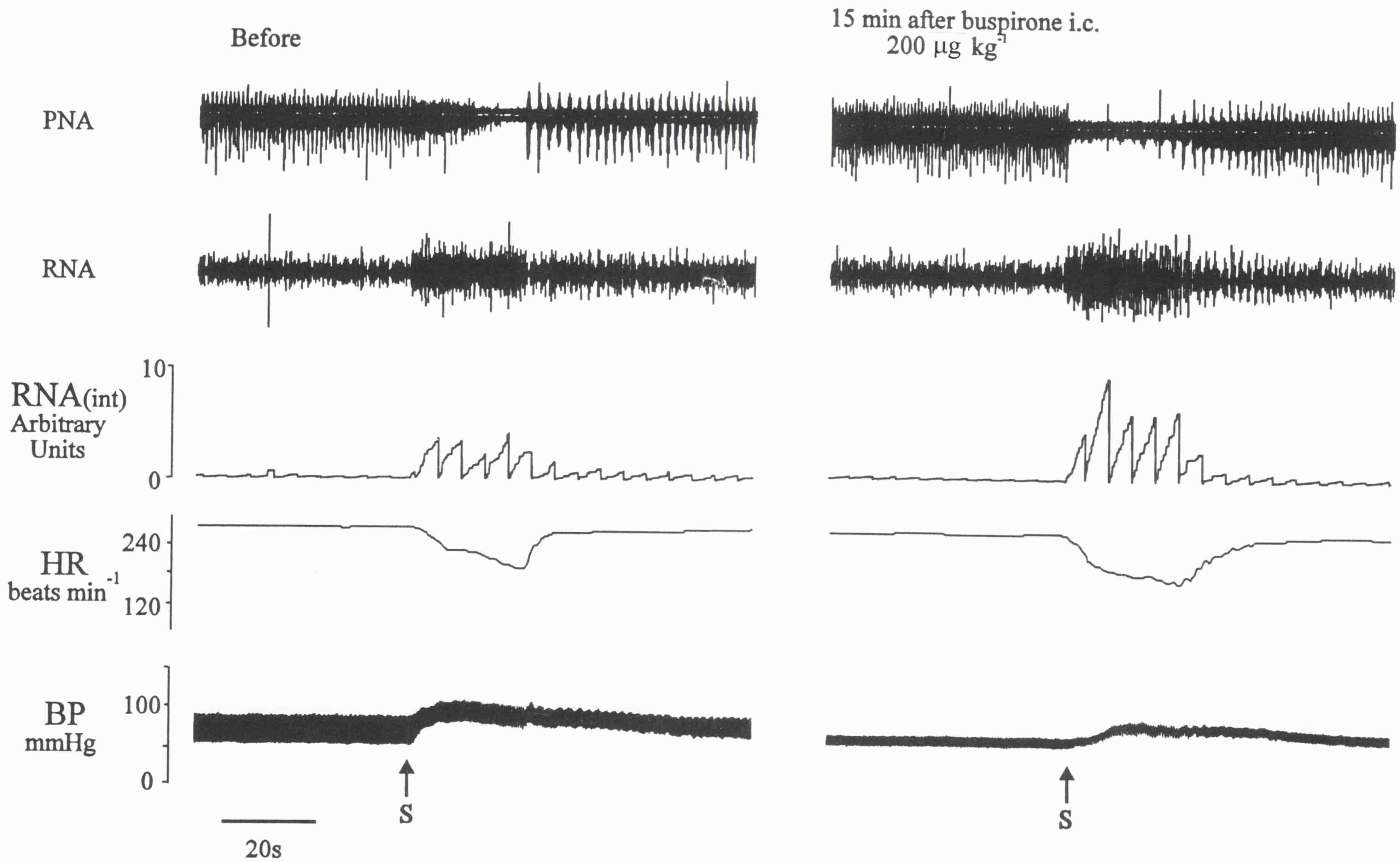

Before
15 min after buspirone i.c.
200 μg kg-1
PNA
RNA
RNA(int)
Arbitrary Units
10
0
HR
beats min-1
240
120
BP
mmHg
100
0
S
S
20s

Figure 3.2

Trace shows the response to smoke delivered to the upper airways, in an atenolol (1 mg kg^{-1}; i.v.) pretreated rabbit, 5 minutes before and 15 minutes after administration of 25 $\mu g\ kg^{-1}$ (+)8-OH-DPAT i.c.

From the top, the traces illustrate phrenic nerve activity (PNA), renal nerve activity (RNA), renal nerve activity integrated with a 5 s time constant (RNA(int)), heart rate (HR) and blood pressure (BP). Each trace is 120 s in duration.

A bolus of smoke was passed through the upper airways at the point marked by an arrow and the letter S.

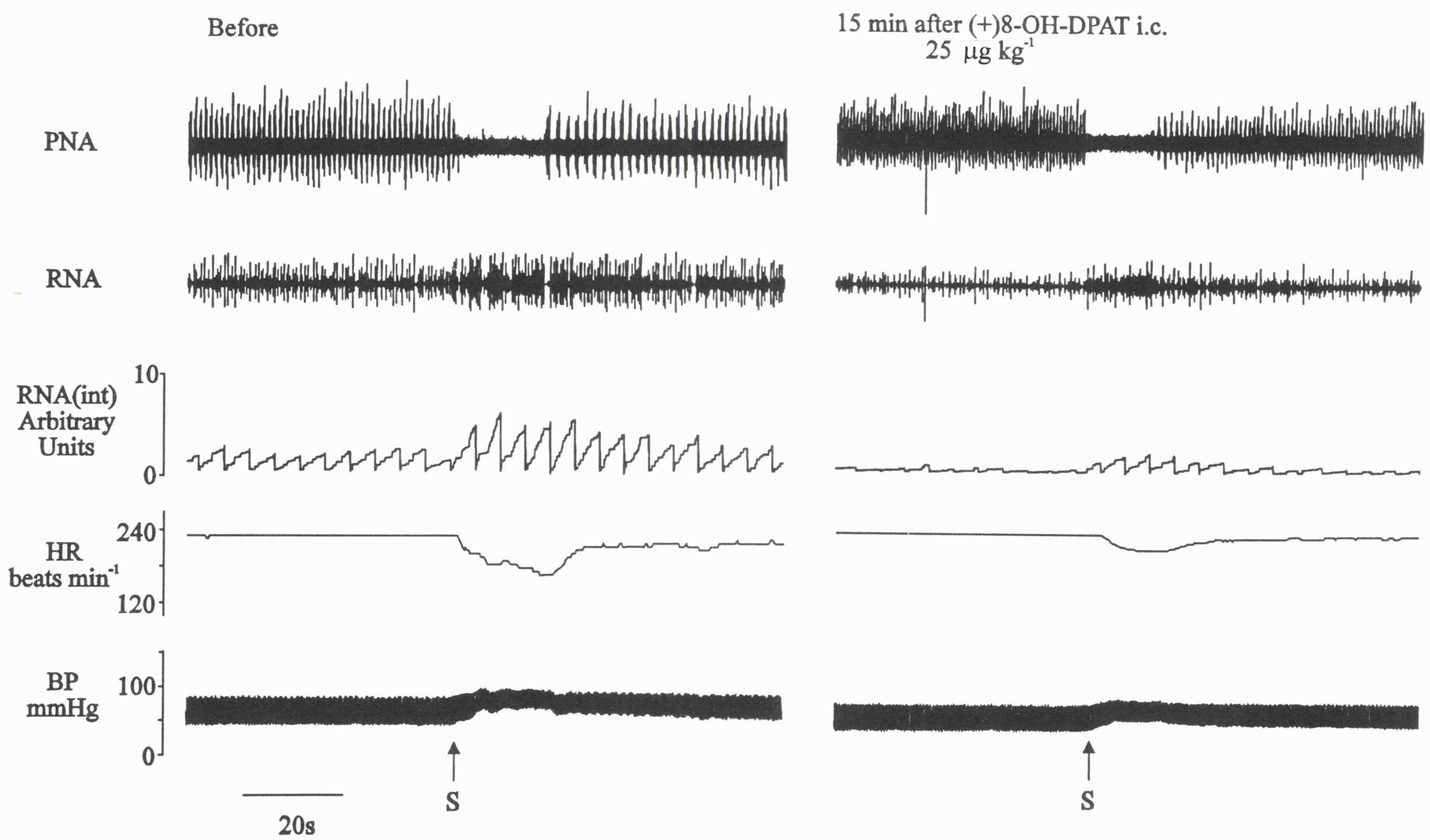

Before
15 min after (+)8-OH-DPAT i.c.
25 μg kg⁻¹
PNA
RNA
RNA(int) Arbitrary Units
10
0
HR beats min⁻¹
240
120
BP mmHg
100
0
S
S
20s

Figure 3.3

Trace shows the response to smoke delivered to the upper airways, in an atenolol (1 mg kg^{-1}; i.v.) pretreated rabbit, 5 min and 15 min after administration of 100 $\mu g\ kg^{-1}$ WAY-100635 i.c.

From the top, the traces illustrate phrenic nerve activity (PNA), renal nerve activity (RNA), renal nerve activity integrated with a 5 s time constant (RNA(int)), heart rate (HR) and blood pressure (BP). Each trace is 120 s in duration.

A bolus of smoke was passed through the upper airways at the point marked by an arrow and the letter S.

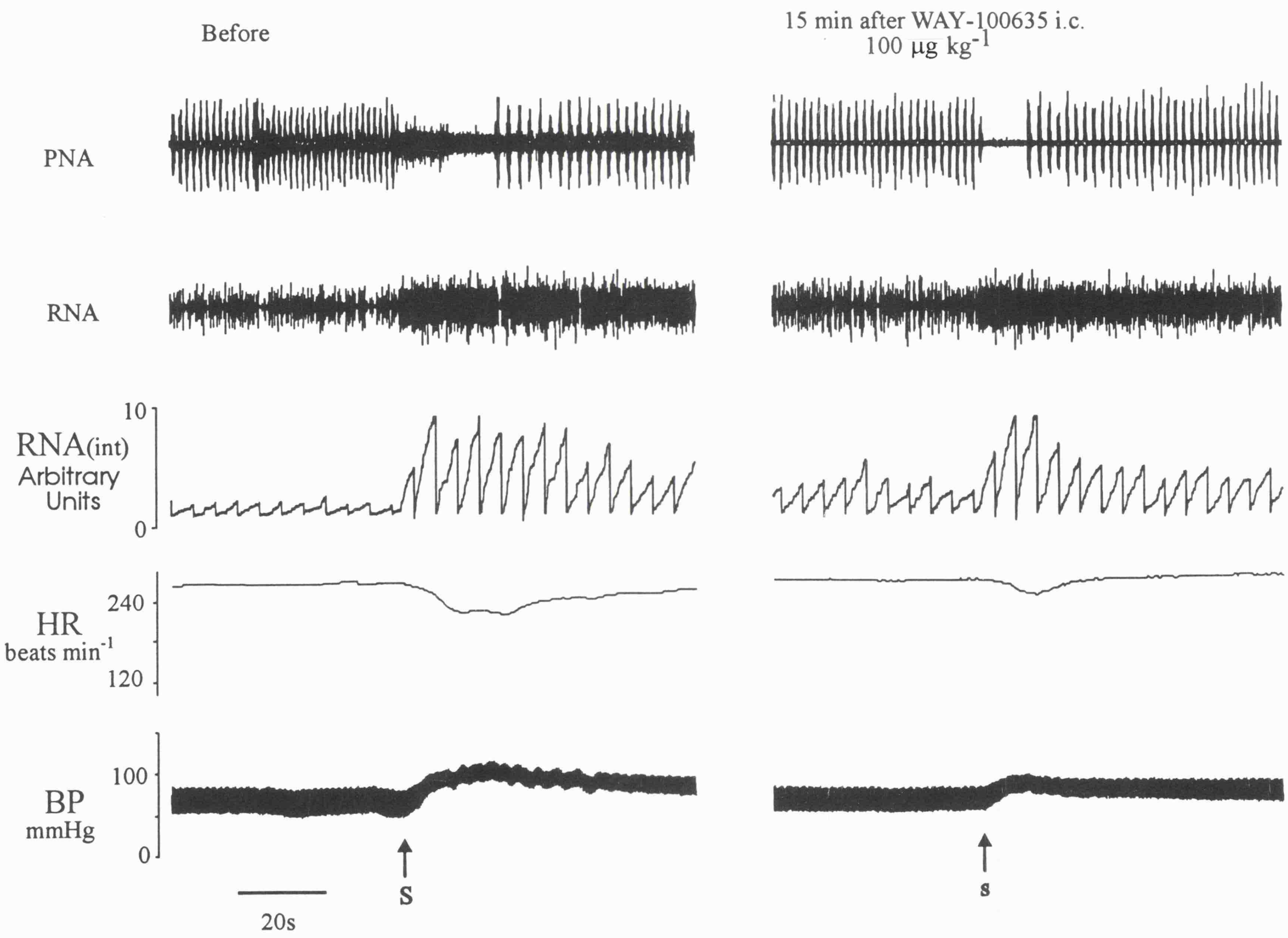

Before
15 min after WAY-100635 i.c.
100 μg kg-1
PNA
RNA
RNA(int)
Arbitrary Units
10
0
HR
beats min-1
240
120
BP
mmHg
100
0
20s
S
s

Figure 3.4 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic nerve burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min after intracisternal (i.c.) injections of buspirone (200 μg kg^{-1}; ■ ; n=5) and saline (20 μl; □ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e.mean. Changes caused by buspirone have been compared with those caused by saline using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

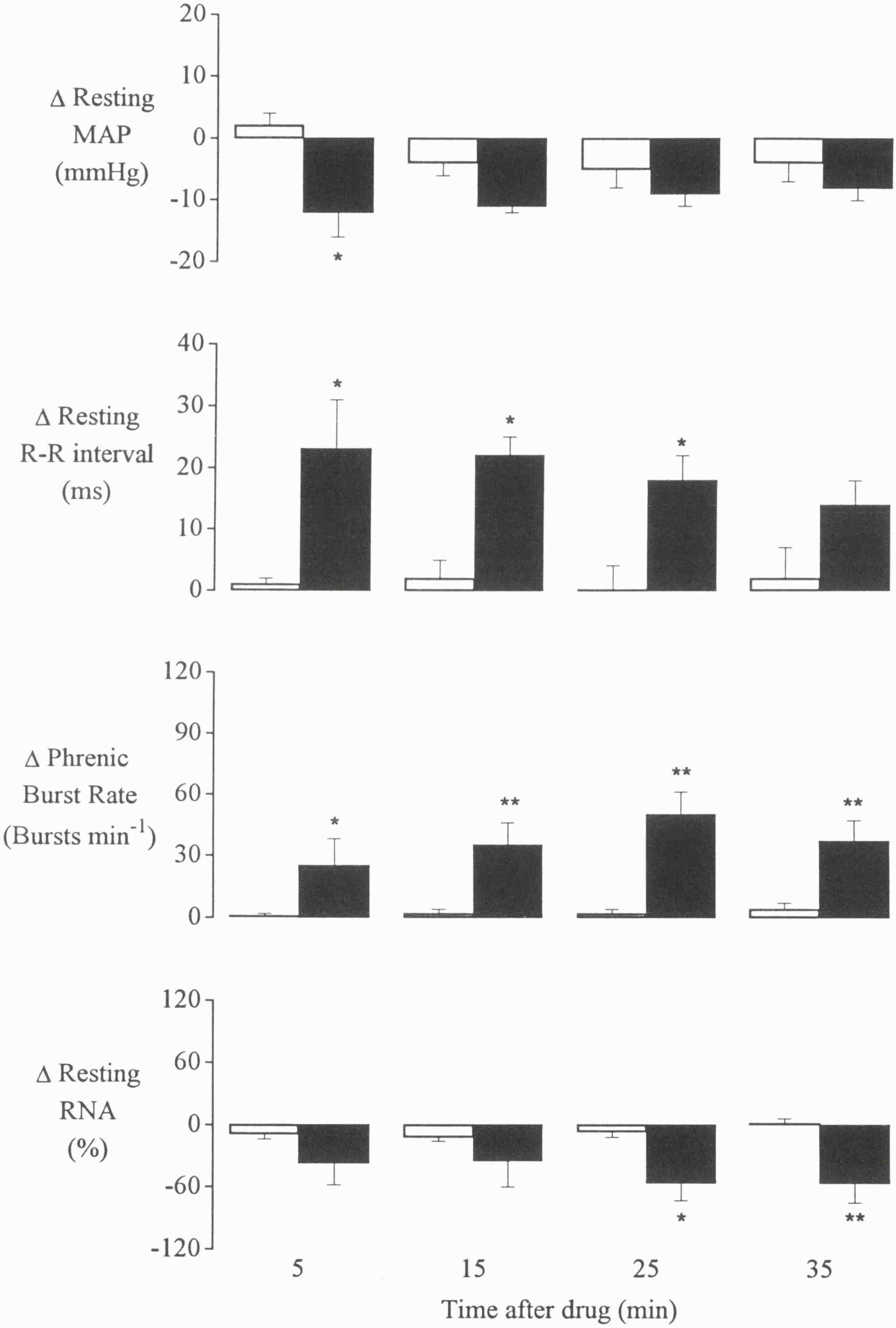
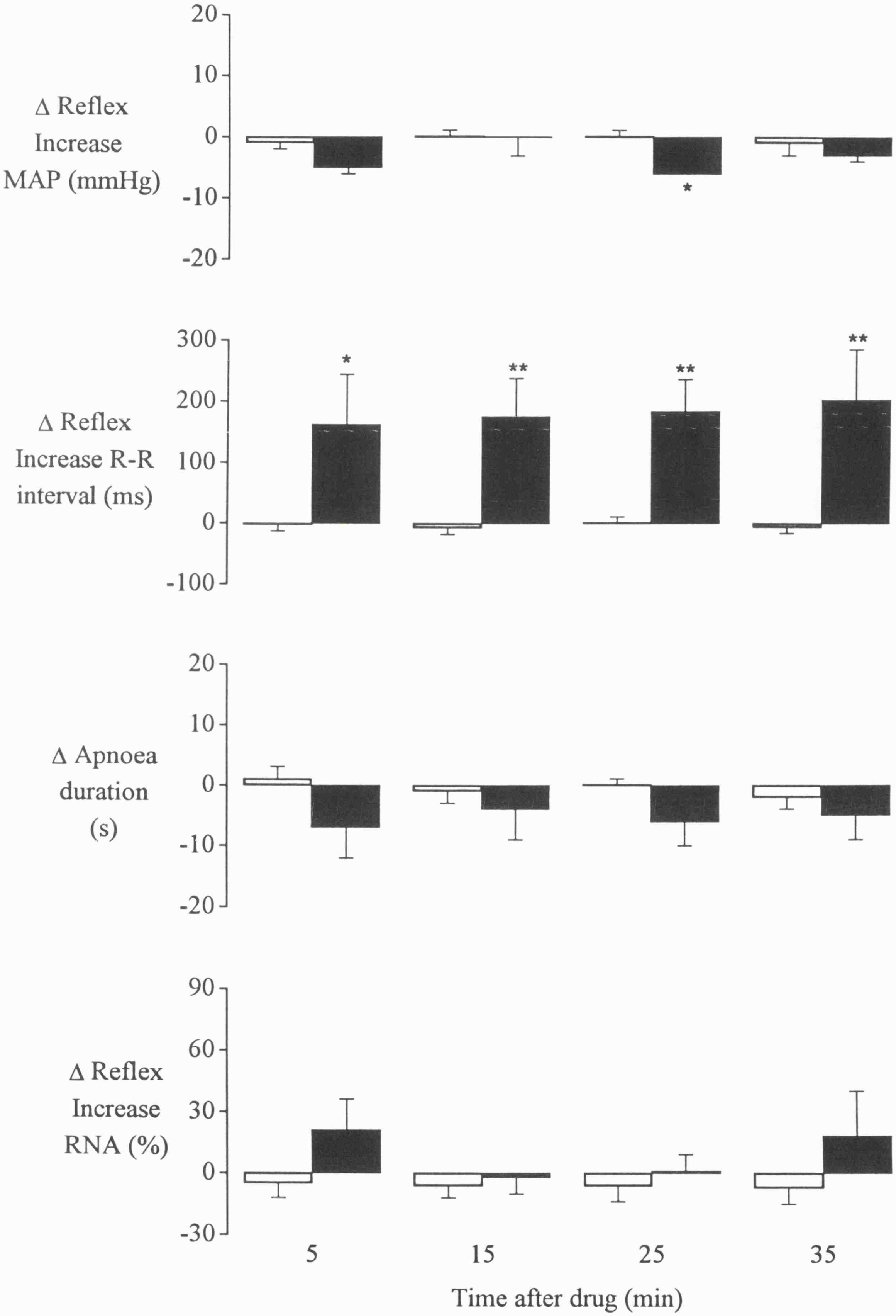

Δ Resting MAP (mmHg)
20
10
0
-10
-20
*
Δ Resting R-R interval (ms)
40
30
20
10
0
*
*
*
Δ Phrenic Burst Rate (Bursts min^{-1})
120
90
60
30
0
*
**
**
**
Δ Resting RNA (%)
120
60
0
-60
-120
*
**
5
15
25
35
Time after drug (min)

Figure 3.5 Anaesthetised, atenolol (i.v. 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in the reflex increases in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min after intracisternal (i.c.) injections of buspirone (200 μg kg^{-1}; ■ ; n=5) and saline (20 μl; □ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by buspirone have been compared with those caused by saline using ANOVA and least significant difference test.
* $p<0.05$; ** $p<0.01$.



20
10
Δ Reflex
Increase
MAP (mmHg)
0
-10
-20
*
300
200
Δ Reflex
Increase R-R
interval (ms)
100
0
-100
*
**
**
**
20
10
Δ Apnoea
duration
(s)
0
-10
-20
90
60
Δ Reflex
Increase
RNA (%)
30
0
-30
5
15
25
35
Time after drug (min)

Figure 3.6 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic nerve burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min after intracisternal (i.c.) injections of saline (20 μl; □ ; n=5), (+)8-OH-DPAT (25 μg kg^{-1}; ■ ; n=5) and n,n-DP-5-CT (50 μg kg^{-1}; ▨ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by drug have been compared with those caused by saline using ANOVA and least significant difference test.
* $p<0.05$; ** $p<0.01$.

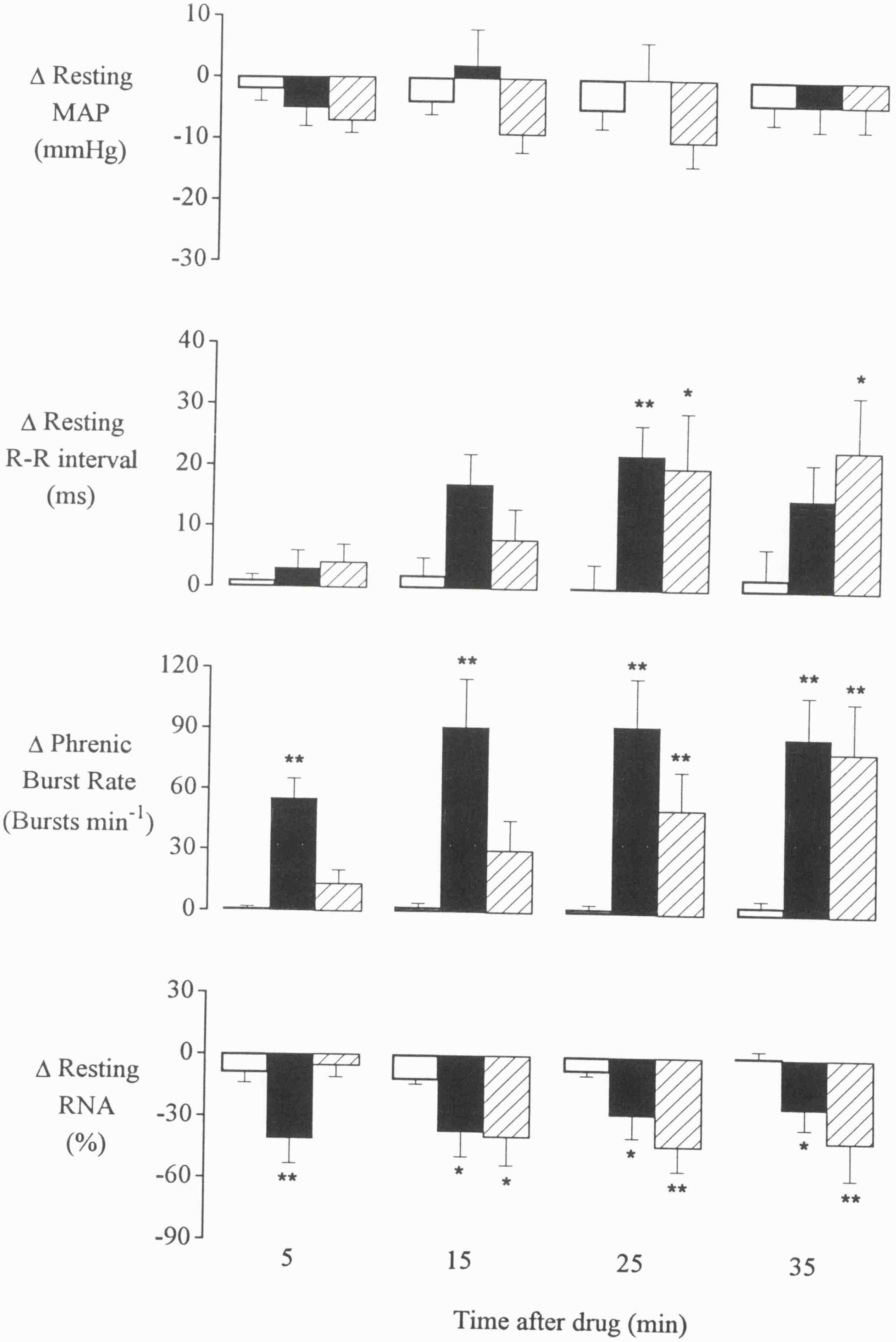

Δ Resting MAP (mmHg)
10
0
-10
-20
-30
Δ Resting R-R interval (ms)
40
30
20
10
0
**
*
*
Δ Phrenic Burst Rate (Bursts min^{-1})
120
90
60
30
0
**
**
**
**
**
**
Δ Resting RNA (%)
30
0
-30
-60
-90
**
*
*
*
**
*
**
5
15
25
35
Time after drug (min)

Figure 3.7 Anaesthetised, atenolol (i.v. 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in the reflex increases in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min after intracisternal (i.c.) injections of saline (20 μl; □ ; n=5), (+)8-OH-DPAT (25 μg kg^{-1}; ■ ; n=5) and n,n-DP-5-CT (50 μg kg^{-1}; ▨ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by drug have been compared with those caused by saline using ANOVA and least significant difference test.

* p<0.05; ** p<0.01.

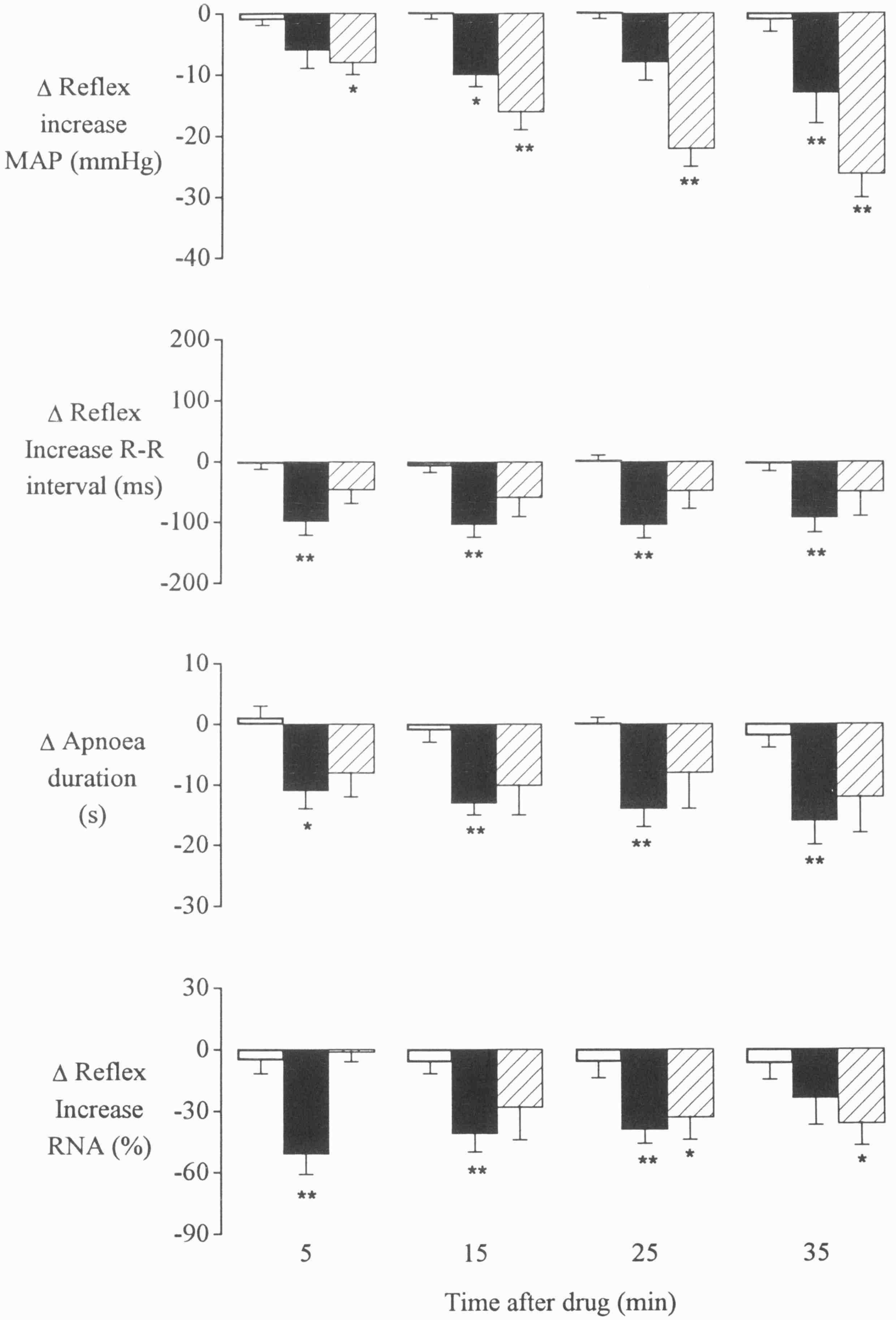
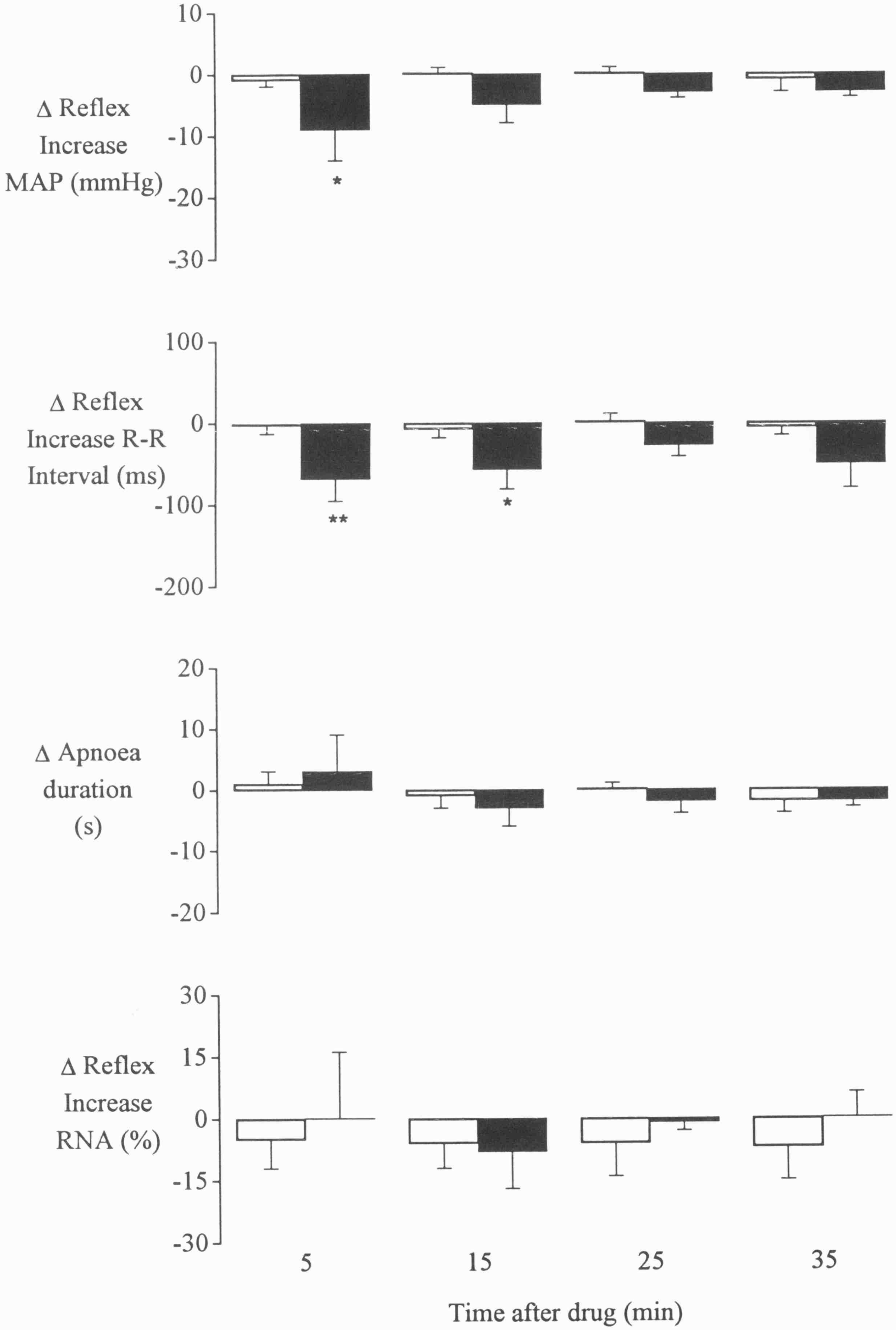

Δ Reflex
increase
MAP (mmHg)
0
-10
-20
-30
-40
Δ Reflex
Increase R-R
interval (ms)
200
100
0
-100
-200
Δ Apnoea
duration
(s)
10
0
-10
-20
-30
Δ Reflex
Increase
RNA (%)
30
0
-30
-60
-90
5
15
25
35
Time after drug (min)

Figure 3.8 Anaesthetised, atenolol (i.v. 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in the reflex increases in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min after intracisternal (i.c.) injections of WAY-100635 (100 μg kg^{-1}; ■ ; n=5) and saline (20 μl; □ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by WAY-100635 have been compared with those caused by saline using ANOVA and least significant difference test. * $p<0.05$; ** $p<0.01$.



10
0
-10
-20
-30
Δ Reflex
Increase
MAP (mmHg)
*
100
0
-100
-200
Δ Reflex
Increase R-R
Interval (ms)
**
*
20
10
0
-10
-20
Δ Apnoea
duration
(s)
30
15
0
-15
-30
Δ Reflex
Increase
RNA (%)
5
15
25
35
Time after drug (min)

Figure 3.9 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic nerve burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min after intracisternal (i.c.) injections of buspirone (200 $\mu g\ kg^{-1}$; ■ ; n=5) and buspirone (200 $\mu g\ kg^{-1}$; ▨ ; n=5) 20 minutes after pretreatment with WAY100635 (i.v.; 100 $\mu g\ kg^{-1}$) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e.mean. Changes caused by buspirone alone have been compared with those caused by buspirone after WAY-100635 pretreatment using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

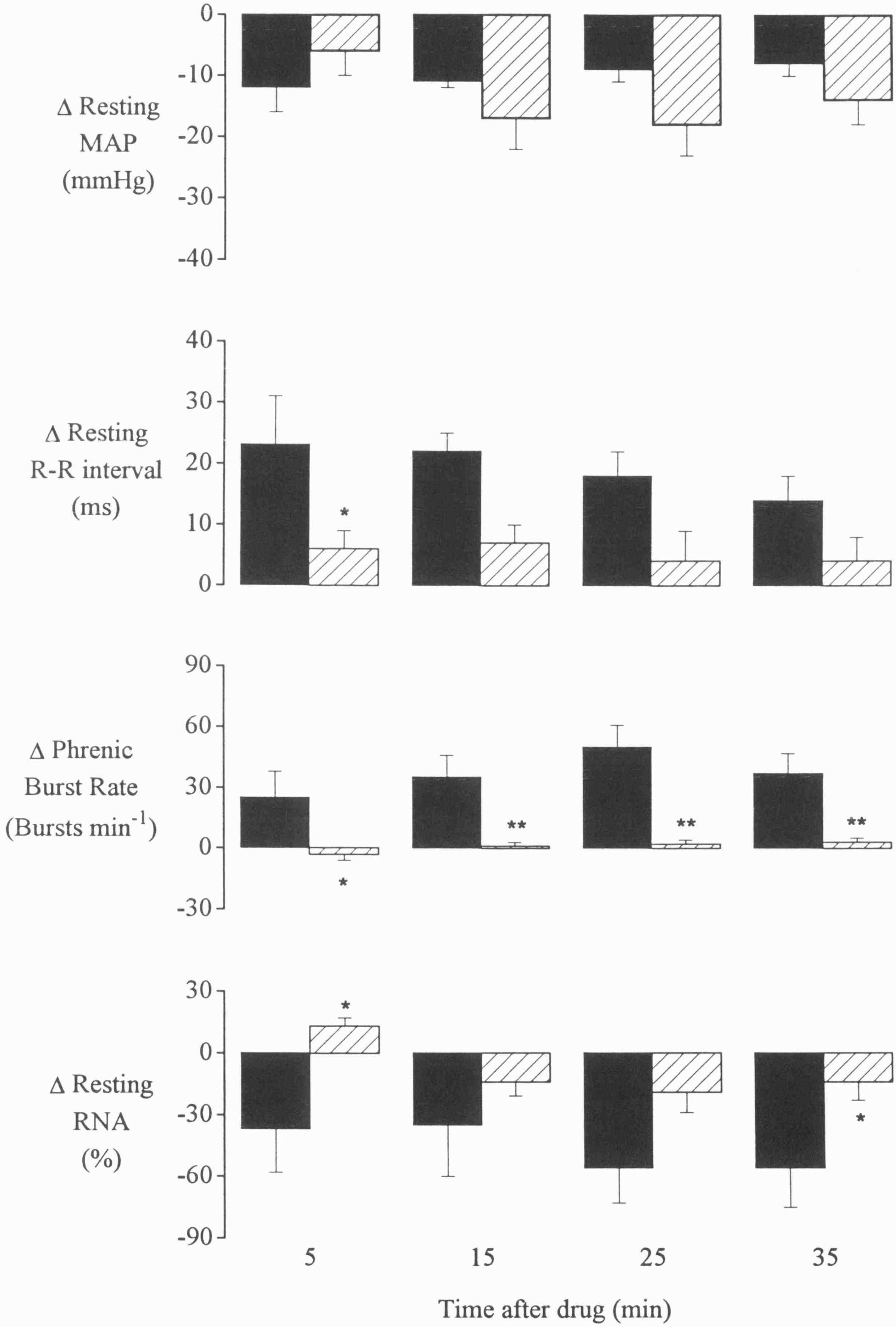
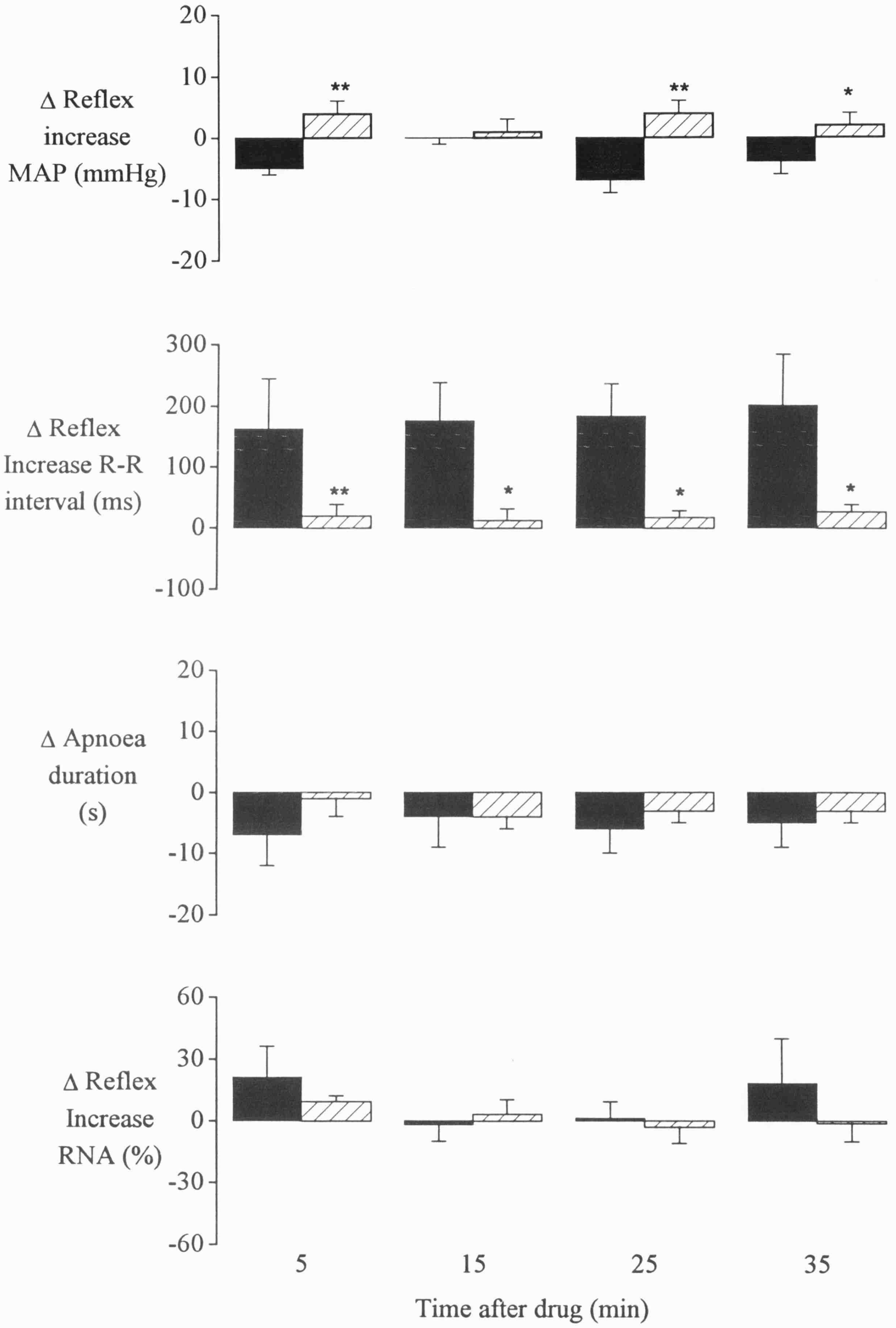

Δ Resting
MAP
(mmHg)
0
-10
-20
-30
-40
Δ Resting
R-R interval
(ms)
40
30
20
10
0
*
Δ Phrenic
Burst Rate
(Bursts min^{-1})
90
60
30
0
-30
*
**
**
**
Δ Resting
RNA
(%)
30
0
-30
-60
-90
*
*
5
15
25
35
Time after drug (min)

Figure 3.10 Anaesthetised, atenolol (i.v. 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in the reflex increases in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min after intracisternal (i.c.) injections of buspirone (200 μg kg^{-1}; ■ ; n=5) and buspirone (200 μg kg^{-1}; ▨ ; n=5) 20 minutes after pretreatment with WAY-100635 (100 μg kg^{-1}; i.v.) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by buspirone have been compared with those caused by saline using ANOVA and least significant difference test.

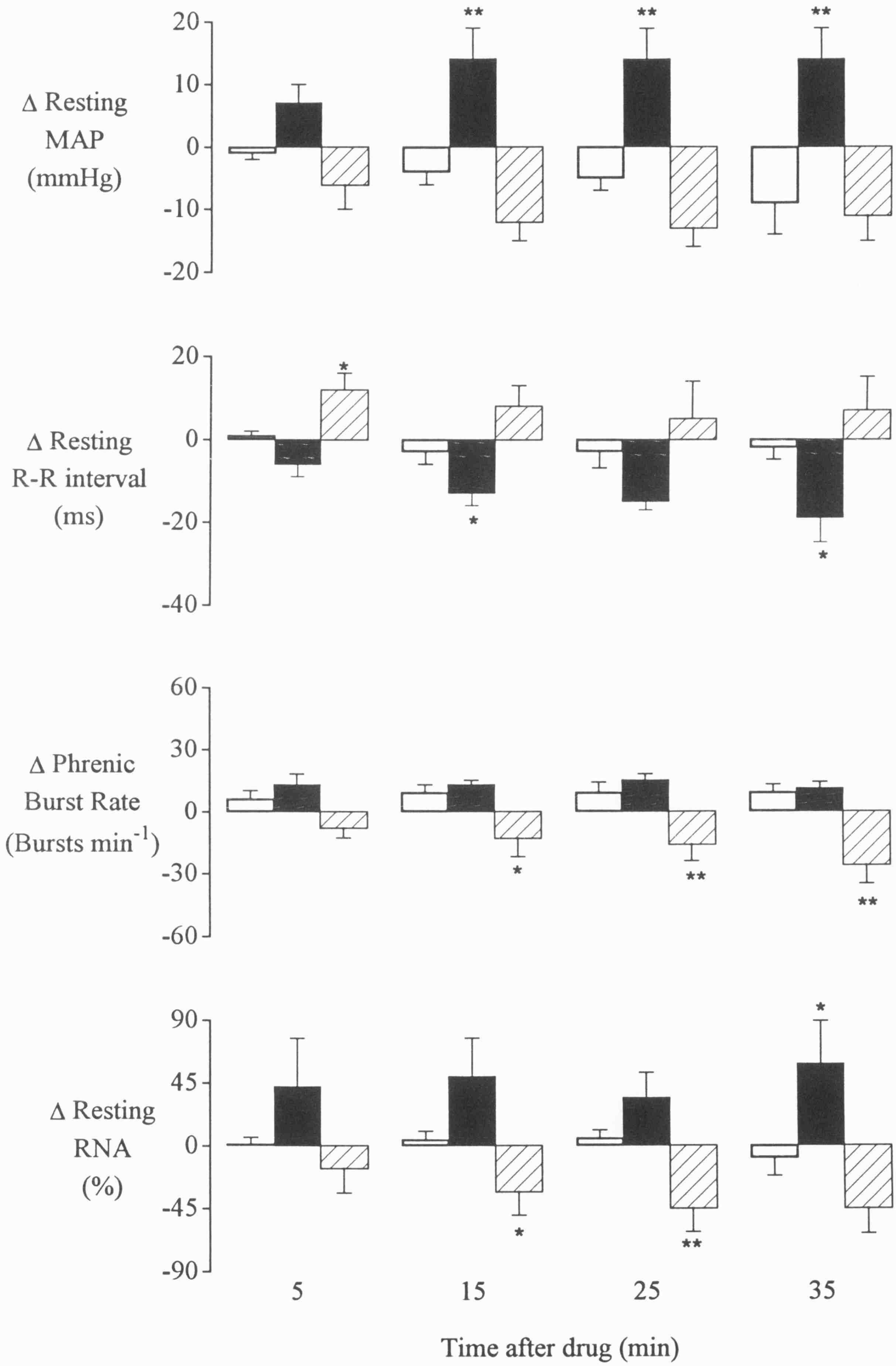
* $p<0.05$; ** $p<0.01$.



Δ Reflex increase MAP (mmHg)
20
10
0
-10
-20
**
**
*
Δ Reflex Increase R-R interval (ms)
300
200
100
0
-100
**
*
*
*
Δ Apnoea duration (s)
20
10
0
-10
-20
Δ Reflex Increase RNA (%)
60
30
0
-30
-60
5
15
25
35
Time after drug (min)

Figure 3.11 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic nerve burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min after intracisternal (i.c.) injections of acidified saline (20 μl; □ ; n=5; pH 2.0), sulpiride (200 μg kg^{-1}; ■ ; n=4) and (-)pindolol (100μg kg^{-1}; ▨ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by drug have been compared with those caused by acidified saline using ANOVA and least significant difference test.

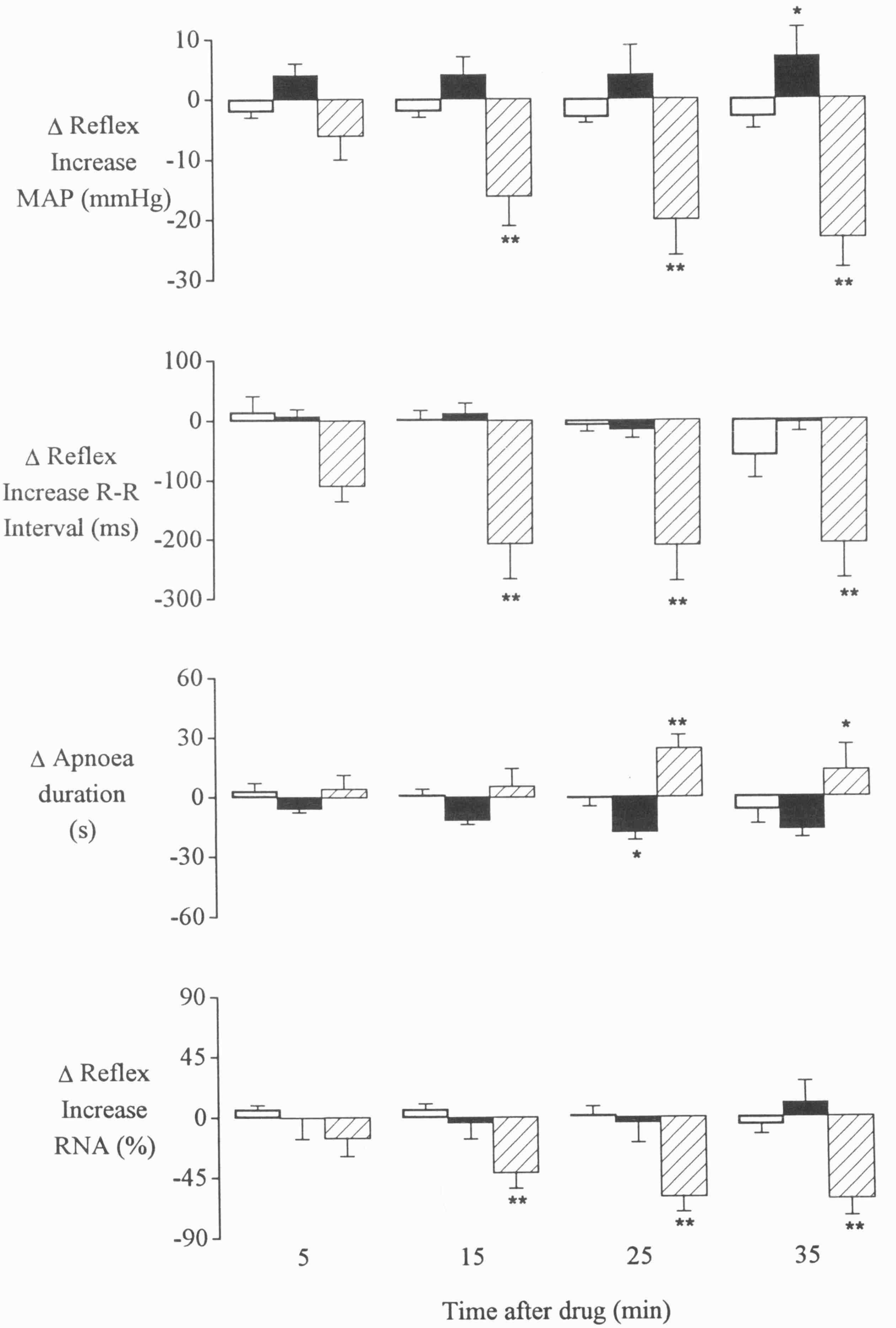
* $p<0.05$; ** $p<0.01$.



Δ Resting MAP (mmHg)
20
10
0
-10
-20
**
**
**
Δ Resting R-R interval (ms)
20
0
-20
-40
*
*
*
Δ Phrenic Burst Rate (Bursts min^{-1})
60
30
0
-30
-60
*
**
**
Δ Resting RNA (%)
90
45
0
-45
-90
*
*
**
5
15
25
35
Time after drug (min)

Figure 3.12 Anaesthetised, atenolol (i.v. 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in the reflex increases in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min after intracisternal (i.c.) injections of acidified saline (20 μl; □ ; n=5; pH 2.0), sulpiride (200 μg kg^{-1}; ■ ; n=4) and (-)pindolol (100 μg kg^{-1}; ▨ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by drug have been compared with those caused by saline using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.



10
0
-10
-20
-30
Δ Reflex
Increase
MAP (mmHg)
*
**
**
**
100
0
-100
-200
-300
Δ Reflex
Increase R-R
Interval (ms)
**
**
**
60
30
0
-30
-60
Δ Apnoea
duration
(s)
**
*
*
90
45
0
-45
-90
Δ Reflex
Increase
RNA (%)
**
**
**
5
15
25
35
Time after drug (min)

Figure 3.13 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic nerve burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min after intracisternal (i.c.) injections of (+)8-OH-DPAT (25 μg kg^{-1}; □ ; n=5), (+)8-OH-DPAT (25 μg kg^{-1}; ■ ; n=4) 20 minutes after pretreatment with GR-127935 (i.v.; 100 μg kg^{-1}) and (+)8-OH-DPAT (25 μg kg^{-1}; ▨ ; n=4) 20 minutes after pretreatment with WAY100635 (i.v.; 100 μg kg^{-1}) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e.mean. Changes caused by (+)8-OH-DPAT alone have been compared with those caused by (+)8-OH-DPAT after pretreatment using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

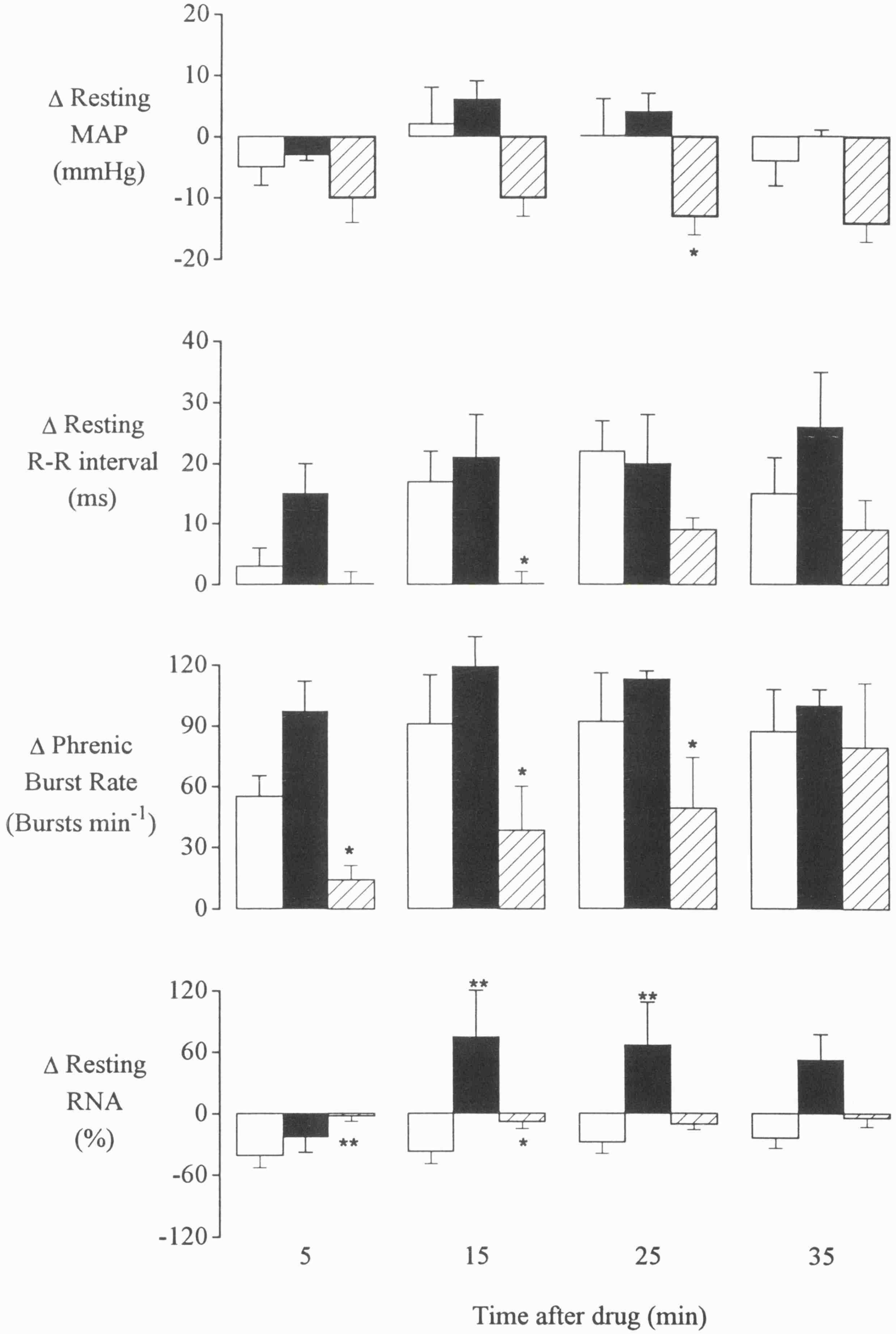
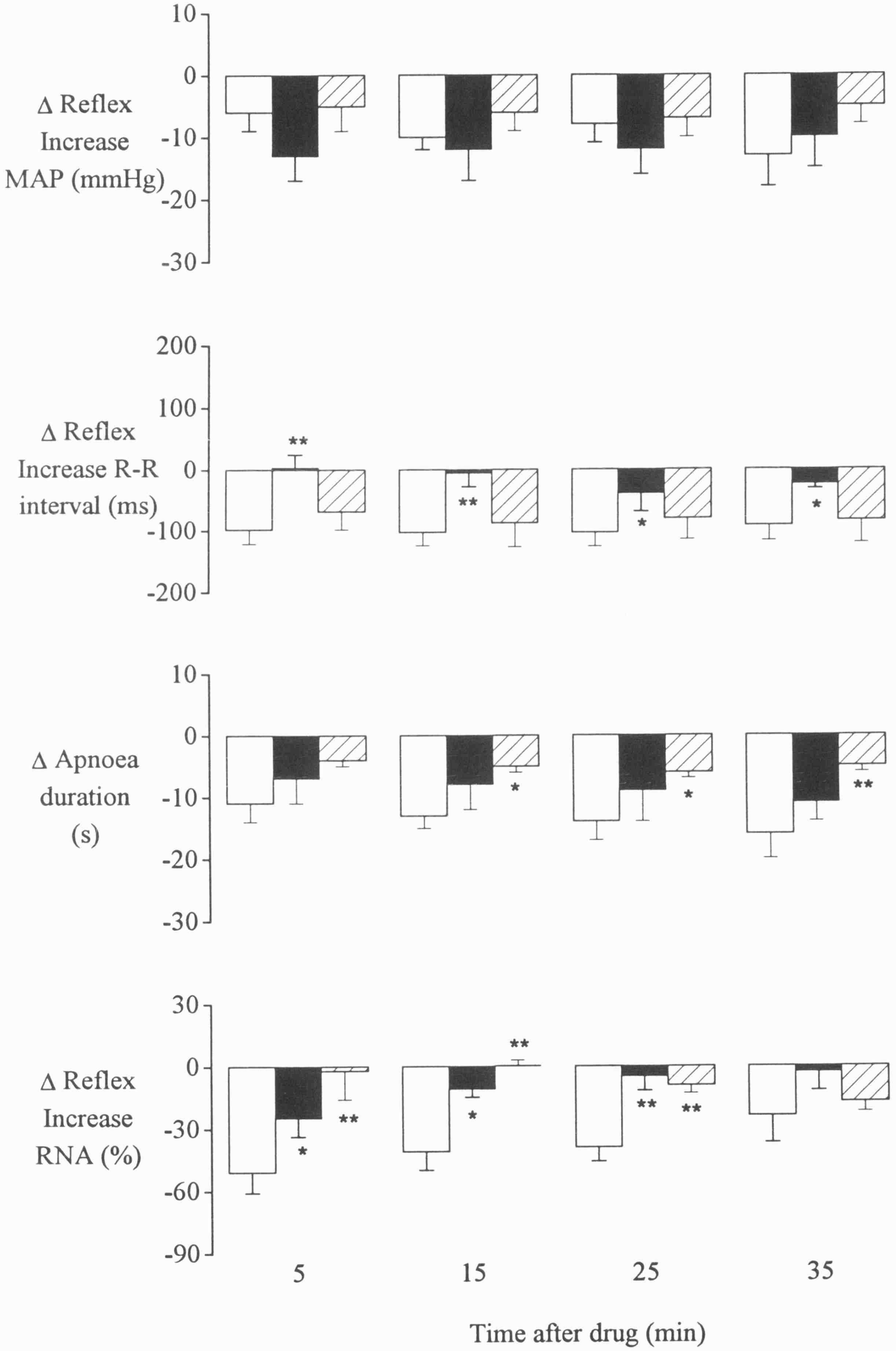

Δ Resting
MAP
(mmHg)
20
10
0
-10
-20
*
Δ Resting
R-R interval
(ms)
40
30
20
10
0
*
Δ Phrenic
Burst Rate
(Bursts min⁻¹)
120
90
60
30
0
*
*
*
Δ Resting
RNA
(%)
120
60
0
-60
-120
**
**
**
*
5
15
25
35
Time after drug (min)

Figure 3.14 Anaesthetised, atenolol (i.v. 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in the reflex increases in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min after intracisternal (i.c.) injections of (+)8-OH-DPAT (25 μg kg^{-1}; □ ; n=5), (+)8-OH-DPAT (25 μg kg^{-1}; ■ ; n=4) 20 minutes after pretreatment with GR-127935 (100 μg kg^{-1}; i.v.) and (+)8-OH-DPAT (25 μg kg^{-1}; ▨ ; n=4) 20 minutes after pretreatment with WAY-100635 (100 μg kg^{-1}; i.v.) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by (+)8-OH-DPAT have been compared with those caused by (+)8-OH-DPAT following pretreatment using ANOVA and least significant difference test.
* $p<0.05$; ** $p<0.01$.


Δ Reflex Increase MAP (mmHg)
Δ Reflex Increase R-R interval (ms)
Δ Apnoea duration (s)
Δ Reflex Increase RNA (%)
5
15
25
35
Time after drug (min)

Effect of 5-HT$_{1D}$ receptor ligands.

Sumatriptan (i.c.).

Resting values.

Administration of sumatriptan (i.c.; 50 μg kg^{-1}; n=5; Table 5.11 in Appendix 5.5) given 20 minutes after pretreatment with WAY-100635 i.v. (100 μg kg^{-1}) caused no significant change to resting R-R interval when compared to i.c. saline. However, sumatriptan did cause small and significant increases in MAP, renal nerve activity and the phrenic burst rate. RNA was increased by 11 ± 4% after 5 min, mean arterial blood pressure by 5 ± 2 mmHg after 15 min and phrenic burst rate by 40 ± 17 bursts min^{-1} after 25 min.

Reflex responses to smoke.

15 min after administration of sumatriptan there was a significant inhibition of the smoke induced reflex increase in R-R interval of -59 ± 14 ms when compared with saline (i.c.; Figure 3.16 and Table 3.10). This was associated only with a significant inhibition of the smoke evoked increase in mean arterial blood pressure of -12 ± 3 mmHg. Apnoea duration and the increase in renal nerve activity were unaffected. However, after 35 min sumatriptan significantly reduced the apnoea duration by -11 ± 5 s. A trace of one of these experiments is shown in Figure 3.15.

GR-127935 (i.c.).

Resting values.

Intracisternal administration of GR-127935 (20 μg kg^{-1}; n=5; Table 5.19 in Appendix 5.5) caused no significant changes to the resting variables when compared with i.c. distilled water (20 μl).

Reflex responses to smoke.

25 min after administration of GR-127935 i.c. there was a significant potentiation of the smoke evoked reflex increase in R-R interval by 47 ± 38 ms (Figure 3.17 and Table 3.11). This was not associated with changes in the other reflex variables. However, the

smoke evoked increase in mean arterial pressure was increased by 4 ± 3 mmHg after 5 min.

GR-127935 (i.v.).

Resting values and Reflex response to smoke.

Administration of GR-127935 i.v. (100 μg kg^{-1}; n=4) had no significant effects, either on the resting variables (Table 5.20 in Appendix 5.5) or the response to the smoke challenge (Table 5.41 in Appendix 5.5). Statistical comparison was made with the effect of 20 μl distilled water i.c. after the same time interval.

Pretreatment with GR-127935 (i.v.) before (+)8-OH-DPAT (i.c.).

Resting values.

Pretreatment with GR-127935 (i.v.; 100 μg kg^{-1}) 20 minutes prior to administration of (+)8-OH-DPAT (i.c.; 25 μg kg^{-1}; n=4; Figure 3.13 and Table 3.8) did not significantly change the effect of (+)8-OH-DPAT on the resting R-R interval, phrenic burst rate or mean arterial blood pressure. There was a significant increase in resting renal nerve activity in the pretreated animals with an increase of 74 ± 46 % after 15 min compared to a decrease of -37 ± 12% in the untreated animals.

Reflex responses to smoke.

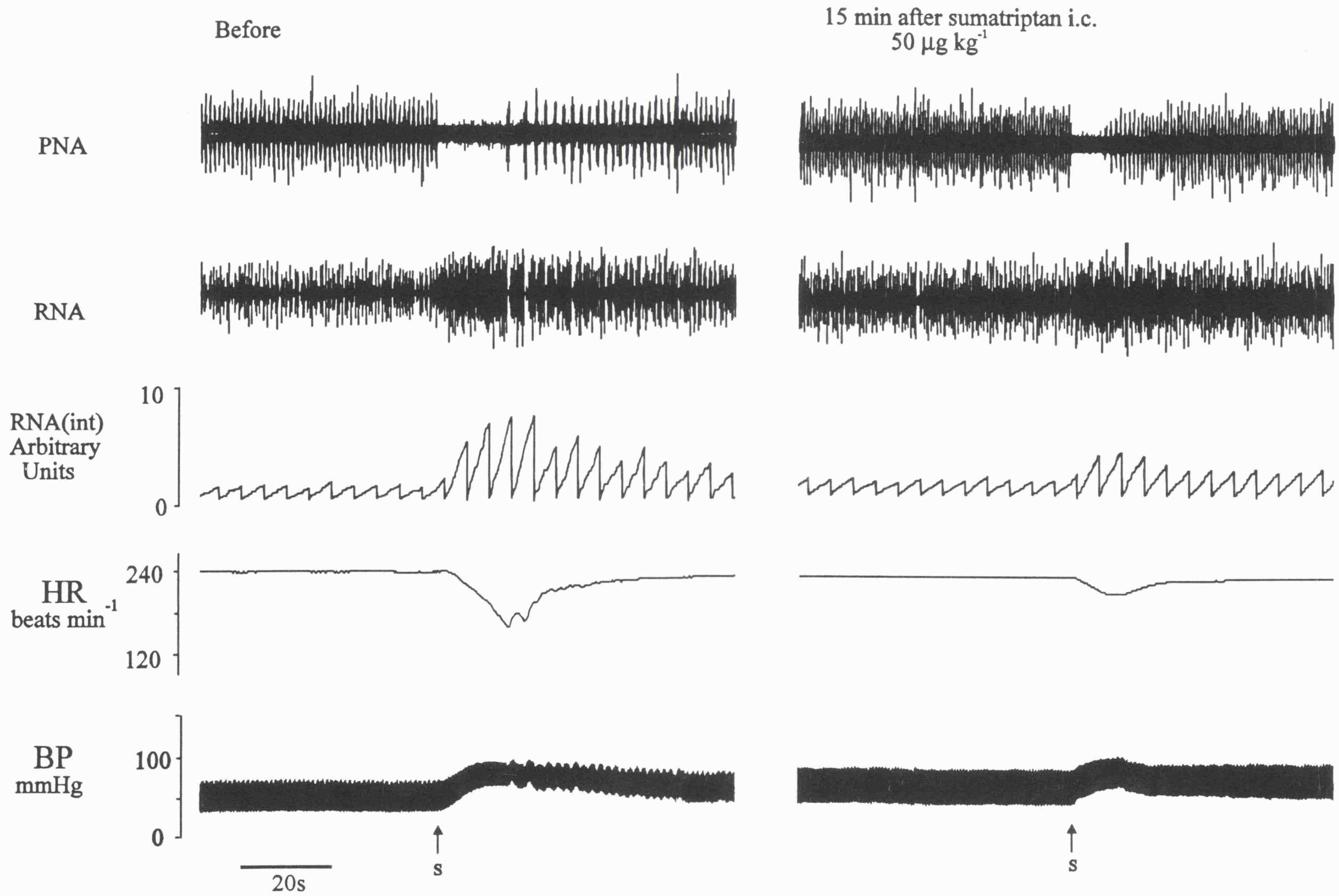
There was a significant difference between the effect of (+)8-OH-DPAT on the smoke evoked reflex increase in R-R interval in untreated and GR-127935 pretreated rabbits (Figure 3.14 and Table 3.11). The inhibition of the reflex bradycardia produced by (+)8-OH-DPAT alone after 5 min was reduced from -98 ± 23 ms to 3 ± 21 ms in the pretreated animals. This was associated with a significant reduction in the (+)8-OH-DPAT mediated inhibition of the smoke evoked increase in renal nerve activity, from -51 ± 10 % after 5 min to -25 ± 9 %. Pretreatment did not significantly alter the effect of 8-OH-DPAT on the apnoea duration, mean arterial pressure rise or increase in R-R interval after 3s.

Figure 3.15

Trace shows the response to smoke delivered to the upper airways in an atenolol (1 mg kg^{-1}; i.v.) pretreated rabbit, 5 minutes before and 15 minutes after administration of 50 $\mu g\ kg^{-1}$ sumatriptan i.c.

From the top, the traces illustrate phrenic nerve activity (PNA), renal nerve activity (RNA), renal nerve activity integrated with a 5 s time constant (RNA(int)), heart rate (HR) and blood pressure (BP). Each trace is 120 s in duration.

A bolus of smoke was passed through the upper airways at the point marked by an arrow and the letter S.



Before
15 min after sumatriptan i.c.
50 µg kg-1
PNA
RNA
RNA(int)
Arbitrary
Units
10
0
HR
beats min-1
240
120
BP
mmHg
100
0
20s
s
s

Figure 3.16 Anaesthetised, atenolol (i.v. 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in the reflex increases in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min after intracisternal (i.c.) injections of saline (20 µl; □ ; n=5) and sumatriptan (50 µg kg^{-1}; ■ ; n=5) 20 minutes after pretreatment with WAY-100635 (100 µg kg^{-1}; i.v.) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by sumatriptan have been compared with those caused by saline using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

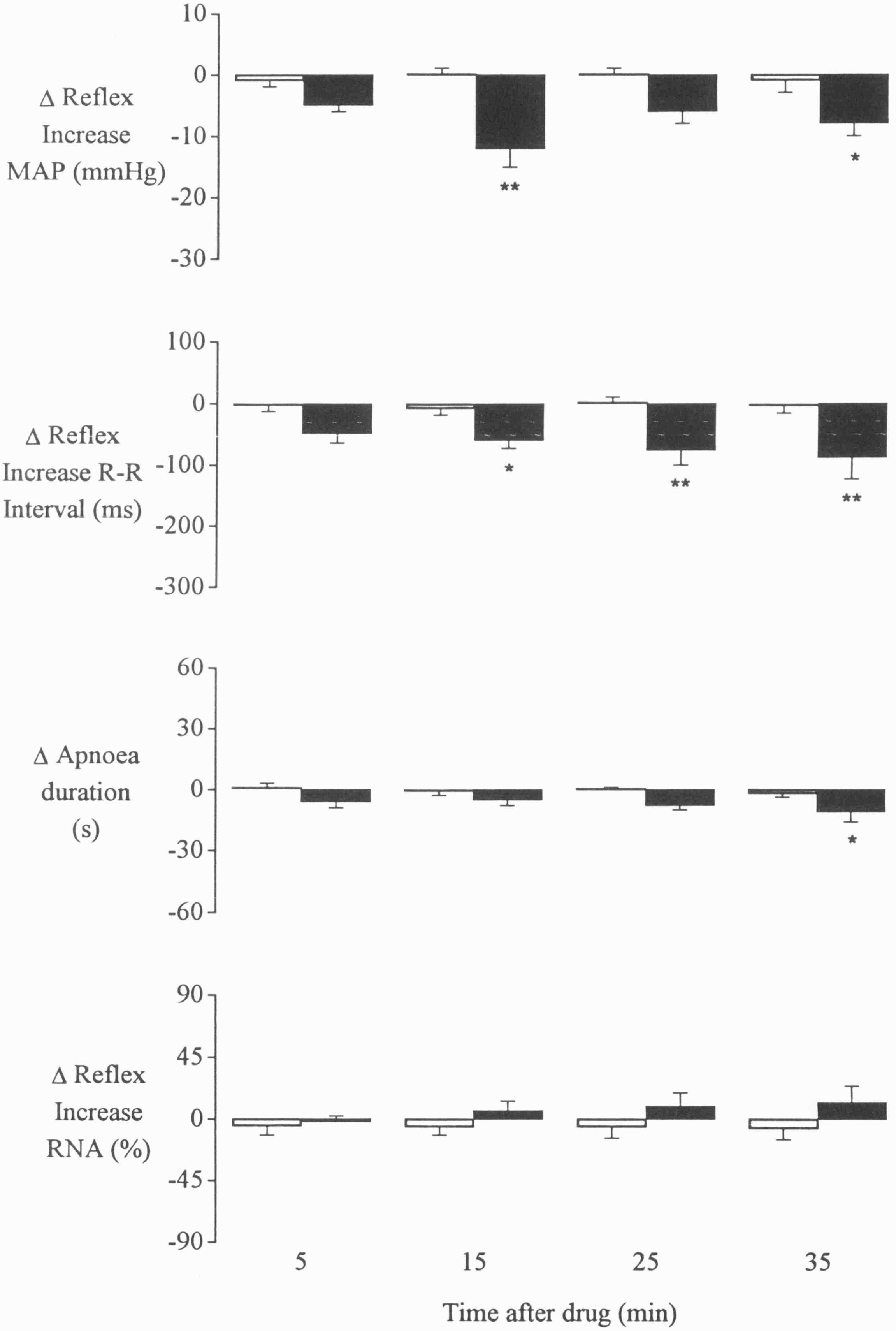
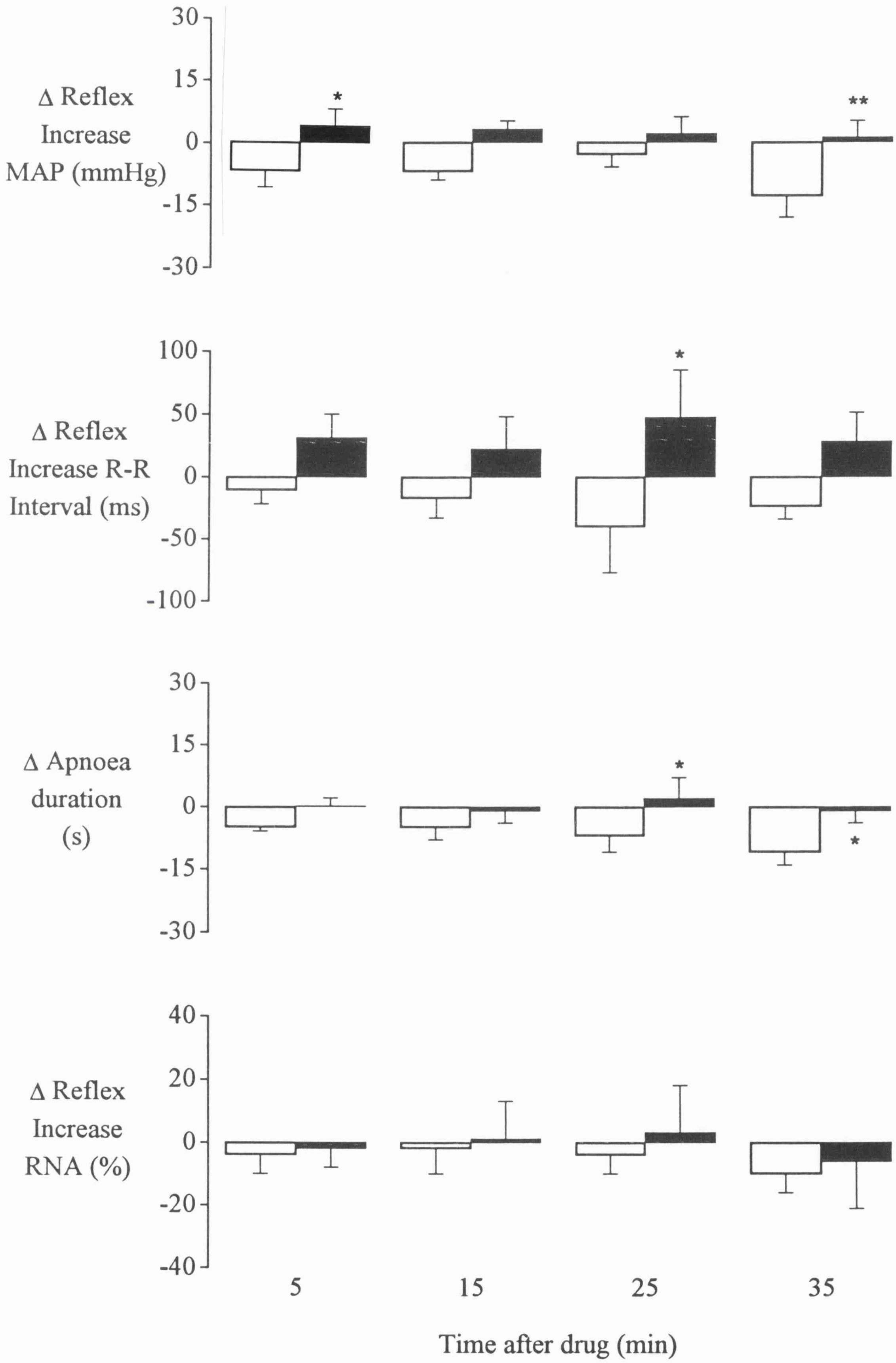

10
0
-10
-20
-30
Δ Reflex
Increase
MAP (mmHg)
**
*
100
0
-100
-200
-300
Δ Reflex
Increase R-R
Interval (ms)
*
**
**
60
30
0
-30
-60
Δ Apnoea
duration
(s)
*
90
45
0
-45
-90
Δ Reflex
Increase
RNA (%)
5
15
25
35
Time after drug (min)

Figure 3.17 Anaesthetised, atenolol (i.v. 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in the reflex increases in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min after intracisternal (i.c.) injections of distilled water (20 μl; □ ; n=5) and GR-127935 (20 μg kg^{-1}; ■ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by GR-127935 have been compared with those caused by distilled water using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.


30
15
0
-15
-30
Δ Reflex
Increase
MAP (mmHg)
*
**
100
50
0
-50
-100
Δ Reflex
Increase R-R
Interval (ms)
*
30
15
0
-15
-30
Δ Apnoea
duration
(s)
*
*
40
20
0
-20
-40
Δ Reflex
Increase
RNA (%)
5
15
25
35
Time after drug (min)

Effect of administration of other ligands.

Granisetron (i.c.).

Resting values.

Administration of granisetron (i.c.; 20 μg kg^{-1}; n=5; Table 5.8 in Appendix 5.5) had no significant effect on baseline MAP, R-R interval, respiratory rate or renal nerve activity.

Reflex responses to smoke.

15 min after administration of granisetron there was a significant inhibition of the smoke evoked increase in R-R interval, which was reduced by -121 ± 55 ms (Figure 3.17 and Table 3.12). This was associated with a significant shortening of the apnoea duration by -7 ± 3 s, but no significant changes in the smoke evoked increase in mean arterial blood pressure or renal nerve activity. A trace of one of these experiments is shown in Figure 3.18.

Granisetron (i.v.).

Resting values and Reflex responses to smoke.

Intravenous administration of granisetron (i.v.; 20 μg kg^{-1}; n=5) caused no significant changes to any of the resting (Table 5.9 in Appendix 5.5) or reflex variables (Figure 3.17 and Table 5.30 in Appendix 5.5).

Mesulergine (i.c.).

Resting values and Reflex responses to smoke.

Administration of mesulergine (i.c.; 200 μg kg^{-1}; n=4) had no effect on any of the resting variables (Table 5.18 in Appendix 5.5). The only significant change to the reflex variables was an increase in the RNA response of 28 ± 13 % after 15 minutes only (Table 5.39 in Appendix 5.5). Statistical comparisons were made with the effects of distilled water (i.c.).

Sulpiride (i.c.).

Resting values.

When sulpiride was administered (i.c.; 200 μg kg^{-1}; n=4) there was a significant reduction in resting R-R interval after 15 min of -13 ± 3 ms (Figure 3.11 and Table 5.15 in Appendix 5.5). This was associated with a significant increase in resting mean arterial blood pressure of 14 ± 5 mmHg. After 35 min there was also a significant increase in resting renal nerve activity, of 58 ± 31 %. There were no significant changes in resting phrenic burst rate.

Reflex responses to smoke.

Sulpiride did not cause a significant change in the smoke evoked reflex increase in R-R interval when compared with the effect of 20 μl acidified saline i.c. after the same time interval (Figure 3.12 and Table 5.36 in Appendix 5.5). There was a significant inhibition of the apnoea duration after 25 min of -18 ± 4 s and the smoke induced increase in mean arterial blood pressure was significantly increased by 7 ± 5 mmHg after 35 min. The increase in renal nerve activity was unaffected.

Figure 3.18

Trace shows the response to smoke delivered to the upper airways in an atenolol (1 mg kg^{-1}; i.v.) pretreated rabbit, 5 minutes before and 15 minutes after administration of 20 $\mu g\ kg^{-1}$ granisetron i.c.

From the top, the traces illustrate phrenic nerve activity (PNA), renal nerve activity (RNA), renal nerve activity integrated with a 5 s time constant (RNA(int)), heart rate (HR) and blood pressure (BP). Each sample is 120 s in duration, the time bar at the bottom of the figure is 20 s long.

A bolus of smoke was passed through the upper airways at the point marked by an arrow and the letter S.

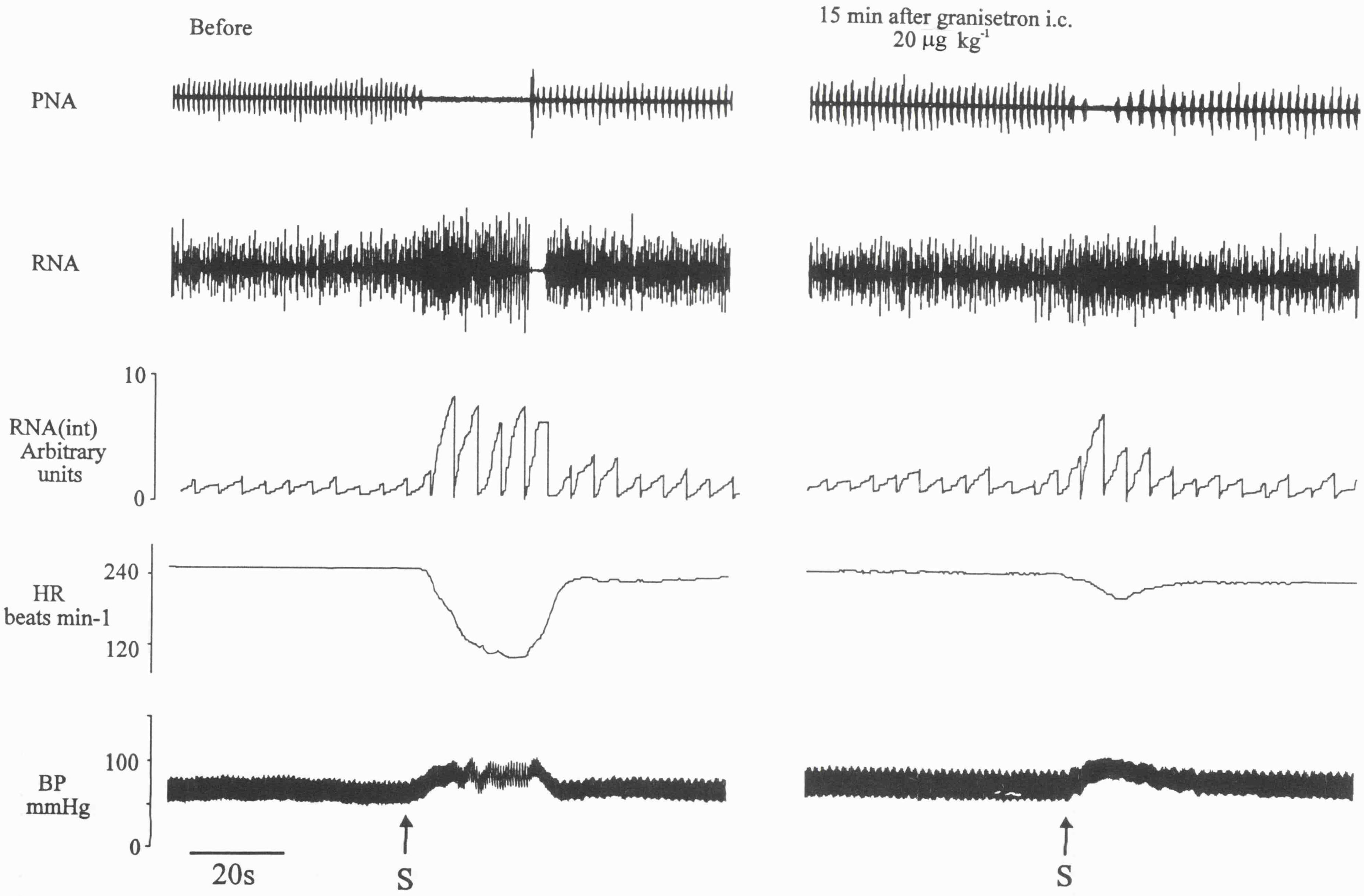
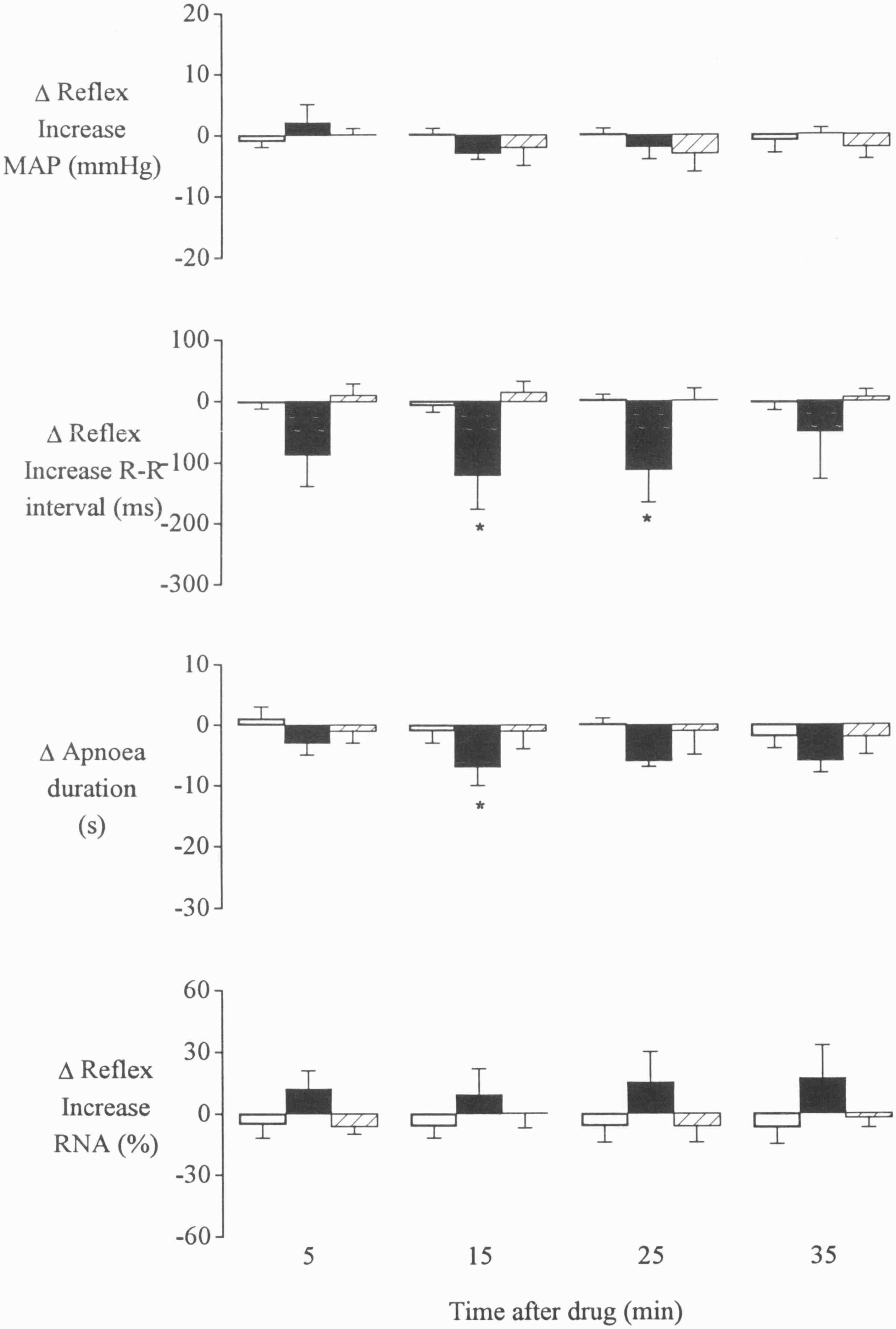

Before
15 min after granisetron i.c.
20 μg kg-1
PNA
RNA
RNA(int)
Arbitrary
units
10
0
HR
beats min-1
240
120
BP
mmHg
100
0
20s
S
S

Figure 3.19 Anaesthetised, atenolol (i.v. 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in the reflex increases in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min after saline (20 μl; □ ; n=5), granisetron (i.c.; 20 μg kg^{-1}; ■ ; n=5) and granisetron (i.v.; 20 μg kg^{-1}; ▨ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by granisetron i.c. or i.v. have been compared with those caused by saline using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.



Δ Reflex Increase MAP (mmHg)
20
10
0
-10
-20
Δ Reflex Increase R-R interval (ms)
100
0
-100
-200
-300
*
*
Δ Apnoea duration (s)
10
0
-10
-20
-30
*
Δ Reflex Increase RNA (%)
60
30
0
-30
-60
5
15
25
35
Time after drug (min)

Table 3.1 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: showing the absolute values (mean ± s.e. mean) of resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min before and 5 min after administration of saline (20 µl i.c.; n=5) or buspirone (200 µg kg^{-1} i.c.; n=5) and thereafter at 10 min intervals over 35 min. The changes (Δ; mean ± s.e. mean) in resting values of the variables are given in parentheses, calculated from the values at 5 min before administration of the test substances. Time matched values of the drug versus vehicle have been compared using ANOVA and least significant difference test.

* p<0.05; ** p<0.01.

Table 3.1

Saline 20 µl i.c. n=5.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	55 ± 3	53 ± 3 **(2 ± 2)**	51 ± 4 **(-4 ± 2)**	50 ± 4 **(-5 ± 3)**	51 ± 5 **(-4 ± 3)**
R-R interval (ms)	243 ± 8	244 ± 8 **(1 ± 1)**	245 ± 8 **(2 ± 3)**	243 ± 7 **(0 ± 4)**	245 ± 8 **(2 ± 5)**
Phrenic burst rate(bursts min^{-1})	50 ± 5	51 ± 5 **(1 ± 1)**	51 ± 5 **(2 ± 2)**	52 ± 5 **(2 ± 2)**	54 ± 6 **(4 ± 3)**
RNA (%)	100	90 ± 5 **(-9 ± 5)**	87 ± 4 **(-12 ± 4)**	93 ± 5 **(-7 ± 5)**	101 ± 5 **(1 ± 5)**

Buspirone 200 µg kg^{-1} i.c. n=5.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	64 ± 6	52 ± 6 **(-12 ± 4*)**	53 ± 5 **(-11 ± 1)**	56 ± 6 **(-9 ± 2)**	57 ± 6 **(-8 ± 2)**
R-R interval (ms)	259 ± 13	282 ± 11* **(23 ± 8*)**	281 ± 11* **(22 ± 3*)**	277 ± 12* **(18 ± 4*)**	273 ± 13 **(14 ± 4)**
Phrenic burst rate(bursts min^{-1})	56 ± 5	73 ± 4 **(25 ± 13*)**	83 ± 4** **(35 ± 11**)**	98 ± 2** **(50 ± 11**)**	85 ± 6** **(37 ± 10**)**
RNA (%)	100	63 ± 21 **(-37 ± 21)**	64 ± 25 **(-35 ± 25)**	44 ± 16* **(-56 ± 17*)**	44 ± 19****(-56 ±19**)**

Table 3.2 Anaesthetised, atenolol (i.v.; 1 mg kg) pretreated, normoxic, spontaneously breathing rabbits: showing the absolute reflex changes (mean ± s.e. mean) in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min before and 5 min after administration of saline (20 μl i.c.; n=5) or buspirone (200 μg kg^{-1} i.c.; n=5) and thereafter at 10 min intervals over 35 min. Changes (Δ; mean ± s.e. mean) in the reflex response of the variables from preinjection (-5 min) values are given. Time matched comparison of drug versus vehicle have been made using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.2 **Saline 20 μl i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	22 ± 4	22 ± 5 **(-1±1)**	22 ± 5 **(0±1)**	23 ± 4 **(0±1)**	21 ± 5 **(-1±2)**
Increase R-R interval (ms)	128 ± 41	124 ± 45 **(-4±9)**	119 ± 46 **(-7±11)**	129 ± 38 **(2±9)**	119 ± 36 **(-6±10)**
3s R-R increase (ms)	7 ± 2	7 ± 2 **(0±1)**	11 ± 6 **(4±4)**	10 ± 5 **(2±4)**	10 ± 6 **(3±5)**
Apnoea duration (s)	25 ± 5	26 ± 5 **(1±2)**	24 ± 4 **(-1±2)**	24 ± 5 **(0±1)**	23 ± 5 **(-2±2)**
RNA (%)	100	97 ± 8 **(-5±7)**	102 ± 4 **(-6±6)**	103 ± 4 **(-6±8)**	102 ± 5 **(-7±8)**

Buspirone 200 μg kg^{-1} i.c. n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	29 ± 1	24 ± 1 **(-5±1)**	29 ± 3 **(-0±3)**	22 ± 1 **(-6±0*)**	25 ± 0 **(-3±1)**
Increase R-R interval (ms)	135 ± 26	324 ± 86* **(162±82*)**	283 ± 83 **(175±63**)**	285 ± 73 **(183±53**)**	318 ± 76* **(201±83**)**
3s R-R increase (ms)	15 ± 4	71 ± 17** **(55±19**)**	72 ± 18** **(57±18**)**	54 ± 21** **(39±18*)**	39 ± 13 **(23±11)**
Apnoea duration (s)	22 ± 5	15 ± 4 **(-7±5)**	17 ± 5 **(-4±5)**	16 ± 3 **(-6±4)**	16 ± 3 **(-5±4)**
RNA (%)	100	121 ± 15 **(21±15)**	98 ± 8 **(-2±8)**	101 ± 8 **(1±8)**	118 ± 22 **(18±22)**

Table 3.3 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: showing the absolute values (mean ± s.e. mean) of resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min before and 5 min after administration of (+)8-OH-DPAT (25 μg kg^{-1} i.c.; n=5) or n,n-DP-5-CT (50 μg kg^{-1} i.c.; n=5) and thereafter at 10 min intervals over 35 min. The changes (Δ; mean ± s.e. mean) in resting values of the variables are given in parentheses, calculated from the values at 5 min before administration of the test substances. Time matched values of the drug versus vehicle have been compared using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.3

(+)8-OH-DPAT 25 μg kg^{-1} i.c. n=5.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	52 ± 7	47 ± 5 **(-5 ± 3)**	53 ± 2 **(2 ± 6)**	51 ± 4 **(0 ± 6)**	48 ± 4 **(-4 ± 4)**
R-R interval (ms)	262 ± 11	265 ± 9 **(3 ± 3)**	279 ± 9** **(17 ± 5)**	284 ± 7****(22 ± 5**)**	277 ± 6****(15 ± 6)**
Phrenic burst rate(bursts min^{-1})	55 ± 3	110 ± 10****(55 ± 10**)**	146 ± 26****(91 ± 24**)**	147 ± 25****(92 ± 24**)**	141 ± 22****(87 ± 21**)**
RNA (%)	100	59 ± 12****(-41 ± 12**)**	63 ± 12***(-37 ± 12*)**	72 ± 11 **(-28 ± 11*)**	76 ± 10***(-24 ± 10*)**

nn-DP-5-CT 50 μg kg^{-1} i.c. n=5.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	61 ± 5	54 ± 4 **(-7 ± 2)**	52 ± 4 **(-9 ± 3)**	51 ± 5 **(-10 ± 4)**	58 ± 8 **(-4 ± 4)**
R-R interval (ms)	266 ± 11	270 ± 13 **(4 ± 3)**	274 ± 14 **(8 ± 5)**	286 ± 17***(20 ± 9*)**	289 ± 17* **(23 ± 9*)**
Phrenic burst rate(bursts min^{-1})	58 ± 5	71 ± 4 **(13 ± 7)**	88 ± 12* **(30 ± 15)**	110 ± 15****(51 ± 19**)**	138 ± 22****(80 ± 25**)**
RNA (%)	100	95 ± 6 **(-5 ± 6)**	61 ± 14* **(-39 ± 14*)**	57 ± 12****(-43 ± 12**)**	60 ± 18****(-40 ± 18**)**

Table 3.4 Anaesthetised, atenolol (i.v.; 1 mg kg) pretreated, normoxic, spontaneously breathing rabbits: showing the absolute reflex changes (mean ± s.e. mean) in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min before and 5 min after administration of (+)8-OH-DPAT (25 $\mu g\ kg^{-1}$ i.c.; n=5) or n,n-DP-5-CT (50 $\mu g\ kg^{-1}$ i.c.; n=5) and thereafter at 10 min intervals over 35 min. Changes (Δ; mean ± s.e. mean) in the reflex response of the variables from preinjection (-5 min) values are given. Time matched comparison of drug versus vehicle have been made using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.4

(+)8-OH-DPAT 25 μg kg^{-1} i.c. n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	23 ± 3	16 ± 4 **(-6±3)**	13 ± 2 **(-10±2*)**	14 ± 3 **(-8±3)**	9 ± 7 **(-13±5**)**
Increase R-R interval (ms)	141 ± 22	43 ± 11 **(-98±23**)**	38 ± 7 **(-103±21**)**	37 ± 7* **(-104±22**)**	49 ± 13 **(-93±24**)**
3s R-R increase (ms)	9 ± 3	16 ± 11 **(7±11)**	10 ± 5 **(1±5)**	11 ± 3 **(2±5)**	14 ± 5 **(5±7)**
Apnoea duration (s)	21 ± 3	10 ± 2** **(-11±3*)**	8 ± 2** **(-13±2**)**	7 ± 2** **(-14±3**)**	5 ± 1** **(-16±4**)**
RNA (%)	100	52 ± 11****(-51±10**)**	59 ± 9** **(-41±9**)**	61 ± 7** **(-39±7**)**	76 ± 13* **(-24±13)**

nn-DP-5-CT 50 μg kg^{-1} i.c. n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	31 ± 1	25 ± 3 **(-8±2*)**	19 ± 4 **(-16±3**)**	11 ± 5* **(-22±3**)**	8 ± 5* **(-26±4**)**
Increase R-R interval (ms)	142 ± 27	97 ± 20 **(-45±24)**	84 ± 25 **(-58±32)**	94 ± 27 **(-48±30)**	93 ± 36 **(-49±41)**
Apnoea duration (s)	26 ± 6	17 ± 5* **(-8±4)**	16 ± 5* **(-10±5)**	17 ± 4* **(-8±6)**	13 ± 4* **(-12±6)**
RNA (%)	100	99 ± 5 **(-1±5)**	72 ± 16** **(-28±16)**	67 ± 11** **(-33±11*)**	64 ± 11** **(-36±11*)**

Table 3.5 Anaesthetised, atenolol (i.v.; 1 mg kg) pretreated, normoxic, spontaneously breathing rabbits: showing the absolute reflex changes (mean ± s.e. mean) in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min before and 5 min after administration of saline (20 μl i.c.; n=5) or WAY-100635 (100 μg kg^{-1} i.c.; n=5) and thereafter at 10 min intervals over 35 min. Changes (Δ; mean ± s.e. mean) in the reflex response of the variables from preinjection (-5 min) values are given. Time matched comparison of drug versus vehicle have been made using ANOVA and least significant difference test. * $p<0.05$; ** $p<0.01$.

Table 3.5

Saline 20 μl i.c. n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	22 ± 4	22 ± 5 **(-1±1)**	22 ± 5 **(0±1)**	23 ± 4 **(0±1)**	21 ± 5 **(-1±2)**
Increase R-R interval (ms)	128 ± 41	124 ± 45 **(-4±9)**	119 ± 46 **(-7±11)**	129 ± 38 **(2±9)**	119 ± 36 **(-6±10)**
Apnoea duration (s)	25 ± 5	26 ± 5 **(1±2)**	24 ± 4 **(-1±2)**	24 ± 5 **(0±1)**	23 ± 5 **(-2±2)**
RNA (%)	100	97 ± 8 **(-5±7)**	102 ± 4 **(-6±6)**	103 ± 4 **(-6±8)**	102 ± 5 **(-7±8)**

WAY-100635 100 μg kg^{-1} i.c. n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	32 ± 6	22 ± 7 **(-9±5*)**	26 ± 4 **(-5±3)**	28 ± 3 **(-3±1)**	29 ± 2 **(-3±1)**
Increase R-R interval (ms)	158 ± 43	91 ± 44 **(-68±27**)**	105 ± 43 **(-57±24*)**	130 ± 31 **(-28±14)**	108 ± 14 **(-51±30)**
Apnoea duration (s)	22 ± 2	25 ± 6 **(3±6)**	19 ± 4 **(-3±3)**	19 ± 2 **(-2±2)**	20 ± 2 **(-2±1)**
RNA (%)	100	100 ± 16 **(0±16)**	92 ± 9 **(-8±9)**	99 ± 2 **(-1±2)**	100 ± 6 **(0±6)**

Table 3.6 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: showing the absolute values (mean ± s.e. mean) of resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min before and 5 min after administration of buspirone (200 μg kg^{-1} i.c.; n=5) or buspirone (200 μg kg^{-1} i.c.; n=5) 20 minutes after pretreatment with WAY-100635 (i.v.; 100 μg kg^{-1}) and thereafter at 10 min intervals over 35 min. The changes (Δ; mean ± s.e. mean) in resting values of the variables are given in parentheses, calculated from the values at 5 min before administration of the test substances. Time matched values of the drug versus drug following pretreatment have been compared using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.6

Buspirone 200 μg kg-1 i.c. n=5.

Time after buspirone (min)	-5	5	15	25	35
MAP (mmHg)	64 ± 6	52 ± 6 **(-12±4)**	53 ± 5 **(-11±1)**	56 ± 6 **(-9±2)**	57 ± 6 **(-8±2)**
R-R interval (ms)	259 ± 13	282 ± 11**(23±8)**	281 ± 11 **(22±3)**	277 ± 12 **(18±4)**	273 ± 13 **(14±4)**
Phrenic burst rate(bursts min-1)	56 ± 5	73 ± 4 **(25±13)**	83 ± 4 **(35±11)**	98 ± 2 **(50±11)**	85 ± 6 **(37±10)**
RNA (%)	100	63 ± 21 **(-37±21)**	64 ± 25 **(-35±25)**	44 ± 16 **(-56±17)**	44 ± 19 **(-56±19)**

Buspirone 200 μg kg-1 i.c. (WAY-100635 100 μg kg-1 i.v. pretreated, 20 minutes previously) n=5.

Time after buspirone (min)	-5	5	15	25	35
MAP (mmHg)	62 ± 6	55 ± 5 **(-7±4)**	45 ± 2 **(-18±5)**	46 ± 3 **(-16±5)**	49 ± 3 **(-13±4)**
R-R interval (ms)	256 ± 6	262 ± 4 **(6±3*)**	263 ± 4 **(7±3)**	260 ± 5 **(4±5)**	260 ± 5 **(4±4)**
Phrenic burst rate(bursts min-1)	55 ± 2	52 ± 3** **(-3±3*)**	56 ± 3** **(1±2**)**	57 ± 4** **(2±2**)**	58 ± 4** **(3±2**)**
RNA (%)	100	113 ± 4* **(13±4*)**	86 ± 7 **(-14±7)**	81 ± 10 **(-19±10)**	86 ± 9* **(-14±9*)**

Table 3.7 Anaesthetised, atenolol (i.v.; 1 mg kg) pretreated, normoxic, spontaneously breathing rabbits: showing the absolute reflex changes (mean ± s.e. mean) in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min before and 5 min after administration of buspirone alone (200 μg kg^{-1} i.c.; n=5) or buspirone (200 μg kg^{-1} i.c.; n=5) 20 minutes after pretreatment with WAY-100635 (100 μg kg^{-1}; i.v.) and thereafter at 10 min intervals over 35 min. Changes (Δ; mean ± s.e. mean) in the reflex response of the variables from preinjection (-5 min) values are given. Time matched comparison of drug versus drug after pretreatment have been made using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.7 **Buspirone 200 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	29 ± 1	24 ± 1 **(-5±1)**	29 ± 3 **(-0±1)**	22 ± 1 **(-7±2)**	25 ± 0 **(-4±2)**
Increase R-R interval (ms)	135 ± 26	324 ± 86 **(162±82)**	283 ± 83 **(175±63)**	285 ± 73 **(183±53)**	318 ± 76 **(201±83)**
3s increase R-R (ms)	15 ± 4	71 ± 17 **(55±19)**	72 ± 18 **(57±18)**	54 ± 21 **(39±18)**	39 ± 13 **(23±11)**
Apnoea duration (s)	22 ± 5	15 ± 4 **(-7±5)**	17 ± 5 **(-4±5)**	16 ± 3 **(-6±4)**	16 ± 3 **(-5±4)**
RNA (%)	100	121 ± 15 **(21±15)**	98 ± 8 **(-2±8)**	101 ± 8 **(1±8)**	118 ± 22 **(18±22)**

Buspirone 200 μg kg^{-1} i.c. **(WAY-100635 100 μg kg^{-1} i.v.** pretreated 20 mins previously) n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	24 ± 3	28 ± 2 **(4±2**)**	26 ± 3 **(1±2)**	28 ± 3* **(4±2**)**	27 ± 3 **(2±2*)**
Increase R-R interval (ms)	62 ± 12	83 ± 25** **(21±19**)**	78 ± 27** **(15±20*)**	82 ± 20* **(20±11*)**	89 ± 21** **(27±12*)**
3s R-R increase (ms)	5 ± 3	5 ± 1** **(0±1**)**	5 ± 1** **(-1±2**)**	5 ± 2** **(0±4*)**	4 ± 0* **(-1±2)**
Apnoea duration (s)	19 ± 3	18 ± 2 **(-1±3)**	16 ± 3 **(-4±2)**	17 ± 2 **(-3±2)**	17 ± 2 **(-3±2)**
RNA (%)	100	109 ± 3 **(9±3)**	103 ± 7 **(3±7)**	97 ± 8 **(-3±8)**	97 ± 10 **(-3±10)**

Table 3.8 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: showing the absolute values (mean ± s.e. mean) of resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min before and 5 min after administration of (+)8-OH-DPAT (25 μg kg^{-1} i.c.; n=4) 20 minutes after pretreatment with GR-127935 (i.v.; 100 μg kg^{-1}) or (+)8-OH-DPAT (25 μg kg^{-1} i.c.; n=4) 20 minutes after pretreatment with WAY-100635 (i.v.; 100 μg kg^{-1}) and thereafter at 10 min intervals over 35 min. The changes (Δ; mean ± s.e. mean) in resting values of the variables are given in parentheses, calculated from the values at 5 min before administration of the test substances. Time matched values of the (+)8-OH-DPAT versus (+)8-OH-DPAT following pretreatment have been compared using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.8

(+)8-OH-DPAT 25 μg kg^{-1} i.c. (**GR-127935 100 μg kg^{-1} i.v.** pretreated 20 minutes previously) n=4.

Time after (+)8-OH-DPAT (min)	-5	5	15	25	35
MAP (mmHg)	46 ± 2	43 ± 1 **(-3±1)**	52 ± 4 **(6±3)**	49 ± 4 **(4±3)**	46 ± 2 **(0±1)**
R-R interval (ms)	265 ± 6	280 ± 7 **(15±5)**	286 ± 7 **(21±7)**	285 ± 6 **(20±8)**	291 ± 7 **(26±9)**
Phrenic burst rate(bursts min^{-1})	66 ± 13	162 ± 27 **(97±15)**	185 ± 25 **(119±15)**	178 ± 15 **(113±4)**	165 ± 17 **(100±8)**
RNA (%)	100	77 ± 15 **(-23±15)**	174±46** **(74±46**)**	166 ± 42** **(66±42*)**	152 ± 25* **(52±25)**

(+)8-OH-DPAT 25 μg kg^{-1} i.c. (**WAY-100635 100 μg kg^{-1} i.v.** pretreated 20 minutes previously) n=4.

Time after (+)8-OH-DPAT (min)	-5	5	15	25	35
MAP (mmHg)	69 ± 4	59* ± 5 **(-10±4)**	59 ± 4 **(-10±3)**	56 ± 3 **(-13±3*)**	56 ± 3 **(-14±3)**
R-R interval (ms)	269 ± 11	269 ± 11 **(0±1)**	271 ± 11 **(0±1*)**	278 ± 10 **(9±2)**	278 ± 11 **(9±5)**
Phrenic burst rate(bursts min^{-1})	59 ± 3	73 ± 8 **(14±7*)**	97 ± 12* **(38±11*)**	108 ± 14 **(49±13*)**	140 ± 14 **(79±16)**
RNA (%)	100	99 ± 6** **(-2±6**)**	93 ± 7* **(-8±7*)**	90 ± 6 **(-10±6)**	97 ± 9 **(-4±9)**

Table 3.9 Anaesthetised, atenolol (i.v.; 1 mg kg) pretreated, normoxic, spontaneously breathing rabbits: showing the absolute reflex changes (mean ± s.e. mean) in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min before and 5 min after administration of (+)8-OH-DPAT (25 $\mu g\ kg^{-1}$ i.c.; n=4) 20 minutes after pretreatment with GR-127935 (100 $\mu g\ kg^{-1}$; i.v.) or (+)8-OH-DPAT (25 $\mu g\ kg^{-1}$ i.c.; n=4) 20 minutes after pretreatment with WAY-100635 (100 $\mu g\ kg^{-1}$; i.v.) and thereafter at 10 min intervals over 35 min. Changes (Δ; mean ± s.e. mean) in the reflex response of the variables from preinjection (-5 min) values are given. Time matched comparison of (+)8-OH-DPAT versus (+)8-OH-DPAT after pretreatment have been made using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.9

(+)8-OH-DPAT 25 μg kg^{-1} i.c. (GR-127935 100 μg kg^{-1} i.v. pretreated 20 mins previously) n=4.

Time after (+)8-OH-DPAT (min)	-5	5	15	25	35
Increase MAP (mmHg)	26 ± 3	13 ± 4 **(-13±4)**	14 ± 4 **(-12±5)**	14 ± 3 **(-12±4)**	16 ± 4 **(-10±5)**
Increase R-R interval (ms)	88 ± 17	91 ± 29* **(3±21**)**	82 ± 25 **(-6±23**)**	48 ± 20 **(-40±30*)**	63 ± 16 **(-25±8*)**
3s R-R increase (ms)	8 ± 2	10 ± 2 **(3±2)**	16 ± 7 **(9±6)**	19 ± 7 **(12±6)**	20 ± 11 **(12±11)**
Apnoea duration (s)	15 ± 4	8 ± 0 **(-7±4)**	6 ± 0 **(-9±4)**	6 ± 1 **(-9±5)**	3 ± 1 **(-11±3)**
RNA (%)	100	75 ± 9* **(-25±9*)**	89 ± 4* **(-11±4*)**	95 ± 7** **(-5±7**)**	97 ± 9 **(-3±9)**

(+)8-OH-DPAT 25 μg kg^{-1} i.c. (WAY-100635 100 μg kg^{-1} i.v. pretreated 20 min previously) n=4.

Time after (+)8-OH-DPAT (min)	-5	5	15	25	35
Increase MAP (mmHg)	19±5	14±5 **(-5±4)**	13±5 **(-6±3)**	12±5 **(-7±3)**	14±5 **(-5±3)**
Increase R-R interval (ms)	114±48	46±32 **(-68±30)**	26±13 **(-87±40)**	34±15 **(-80±35)**	30±16 **(-84±37)**
3s R-R increase (ms)	15±9	5±4 **(-10±11)**	10±7 **(-5±12)**	7±5 **(-8±11)**	11±6 **(-4±13)**
Apnoea duration (s)	19±2	15±3 **(-4±1)**	14±3 **(-5±1*)**	13±2 **(-6±1*)**	14±2 **(-5±1**)**
RNA (%)	100	98±14 **(-2±14**)**	100±3 **(0±3**)**	91±4 **(-9±4**)**	83±5 **(-17±5)**

Table 3.10 Anaesthetised, atenolol (i.v.; 1 mg kg) pretreated, normoxic, spontaneously breathing rabbits: showing the absolute reflex changes (mean ± s.e. mean) in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min before and 5 min after administration of saline (20 μl i.c.; n=5) or sumatriptan (50 μg kg^{-1} i.c.; n=5) 20 minutes after pretreatment with WAY-100635 (i.v.; 100 μg kg^{-1})and thereafter at 10 min intervals over 35 min. Changes (Δ; mean ± s.e. mean) in the reflex response of the variables from preinjection (-5 min) values are given. Time matched comparison of drug versus vehicle have been made using ANOVA and least significant difference test.
* $p<0.05$; ** $p<0.01$.

Table 3.10

Saline 20 μl i.c. n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	22 ± 4	22 ± 5 **(-1±1)**	22 ± 5 **(0±1)**	23 ± 4 **(0±1)**	21 ± 5 **(-1±2)**
Increase R-R interval (ms)	128 ± 41	124 ± 45 **(-4±9)**	119 ± 46 **(-7±11)**	129 ± 38 **(2±9)**	119 ± 36 **(-6±10)**
Apnoea duration (s)	25 ± 5	26 ± 5 **(1±2)**	24 ± 4 **(-1±2)**	24 ± 5 **(0±1)**	23 ± 5 **(-2±2)**
RNA (%)	100	97 ± 8 **(-5±7)**	102 ± 4 **(-6±6)**	103 ± 4 **(-6±8)**	102 ± 5 **(-7±8)**

Sumatriptan 50 μg kg^{-1} i.c. (WAY-100635 100 μg kg^{-1} i.v. pretreated 20 mins previously) n=5.

Time after sumatriptan (min)	-5	5	15	25	35
Increase MAP (mmHg)	28 ± 3	23 ± 3 **(-5±1)**	16 ± 2 **(-12±3**)**	22 ± 3 **(-6±2)**	19 ± 2 **(-8±2*)**
Increase R-R interval (ms)	131 ± 33	82 ± 28 **(-48±16)**	72 ± 23 **(-59±14*)**	55 ± 11 **(-76±24**)**	43 ± 10 **(-88±35**)**
Apnoea duration (s)	19 ± 4	13 ± 5 **(-6±3)**	13 ± 5 **(-5±3)**	11 ± 4* **(-8±2)**	8 ± 4* **(-11±5*)**
RNA (%)	100	98 ± 4 **(-2±4)**	106 ± 7 **(6±7)**	109 ± 10 **(9±10)**	112 ± 12 **(12±12)**

Table 3.11 Anaesthetised, atenolol (i.v.; 1 mg kg) pretreated, normoxic, spontaneously breathing rabbits: showing the absolute reflex changes (mean ± s.e. mean) in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min before and 5 min after administration of distilled water (20 μl i.c.; n=5) or GR-127935 (20 μg kg^{-1} i.c.; n=5) and thereafter at 10 min intervals over 35 min. Changes (Δ; mean ± s.e. mean) in the reflex response of the variables from preinjection (-5 min) values are given. Time matched comparison of drug versus vehicle have been made using ANOVA and least significant difference test. * $p<0.05$; ** $p<0.01$.

Table 3.11

Distilled water 20 μl i.c. n=4.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	32 ± 3	25 ± 2 **(-7±4)**	28 ± 2 **(-7±2)**	29 ± 1 **(-3±3)**	19 ± 6 **(-13±5)**
Increase R-R interval (ms)	90 ± 53	79 ± 60 **(-11±11)**	73 ± 37 **(-17±16)**	50 ± 17 **(-40±37)**	66 ± 43 **(-24±10)**
Apnoea duration (s)	18 ± 6	12 ± 6 **(-5±1)**	13 ± 4 **(-5±3)**	11 ± 2 **(-7±4)**	7 ± 4 **(-11±3)**
RNA (%)	100	97 ± 6 **(-4±6)**	98 ± 8 **(-2±8)**	96 ± 6 **(-4±6)**	90 ± 6 **(-10±6)**

GR-127935 20 μg kg^{-1} i.c. n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	23 ± 2	27 ± 4 **(4±3*)**	26 ± 3 **(3±2)**	25 ± 4 **(2±4)**	24 ± 5 **(1±4**)**
Increase R-R interval (ms)	56 ± 15	88 ± 32 **(31±19)**	78 ± 36 **(22±26)**	101 ± 50 **(47±38*)**	84 ± 36 **(28±23)**
Apnoea duration (s)	10 ± 2	11 ± 3 **(0±2)**	9 ± 4 **(-1±3)**	12 ± 7 **(2±5*)**	10 ± 4 **(-1±3*)**
RNA (%)	100	99 ± 6 **(-1±6)**	101 ± 12 **(1±12)**	103 ± 15 **(3±15)**	94 ± 15 **(-6±15)**

Table 3.12 Anaesthetised, atenolol (i.v.; 1 mg kg) pretreated, normoxic, spontaneously breathing rabbits: showing the absolute reflex changes (mean ± s.e. mean) in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min before and 5 min after administration of saline (i.c.; 20 μl; n=5), granisetron (i.c.; 20 μg kg^{-1}; n=5) or granisetron (i.v.; 20 μg kg^{-1}; n=5) and thereafter at 10 min intervals over 35 min. Changes (Δ; mean ± s.e. mean) in the reflex response of the variables from preinjection (-5 min) values are given. Time matched comparison of drug versus vehicle have been made using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.12

Granisetron 20 μg kg^{-1} i.c. n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	28 ± 2	30 ± 3 **(2±3)**	24 ± 2 **(-3±1)**	26 ± 2 **(-2±2)**	28 ± 2 **(0±1)**
Increase R-R interval (ms)	198 ± 53	110 ± 46 **(-88±51)**	77 ± 32 **(-121±55*)**	86 ± 29 **(-112±53*)**	147 ± 56 **(-51±77)**
Apnoea duration (s)	19 ± 2	16 ± 3 **(-3±2)**	11 ± 2* **(-7±3*)**	13 ± 2* **(-6±2)**	13 ± 3 **(-6±2)**
RNA (%)	100	112 ± 9 **(12±9)**	109 ± 13 **(9±13)**	115 ± 15 **(15±15)**	117 ± 16 **(17±16)**

Granisetron 20 μg kg^{-1} i.v. n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	30 ± 4	30 ± 4 **(0±1)**	28 ± 5 **(-2±3)**	27 ± 5 **(-3±3)**	28 ± 4 **(-2±2)**
Increase R-R interval (ms)	79 ± 27	88 ± 40 **(9±19)**	93 ± 40 **(14±18)**	80 ± 38 **(1±19)**	84 ± 34 **(5±13)**
Apnoea duration (s)	21 ± 6	20 ± 7 **(-1±2)**	20 ± 9 **(-1±3)**	19 ± 10 **(-1±4)**	19 ± 9 **(-2±3)**
RNA (%)	100	94 ± 4 **(-6±4)**	100 ± 7 **(0±7)**	94 ± 8 **(-6±8)**	98 ± 5 **(-2±5)**

Table 3.13 Summary of results obtained in spontaneously breathing, normoxic rabbits.

Resting. Effects of drugs on baseline R-R interval (R-R), phrenic nerve rate (PNA rate), renal nerve activity (RNA) and mean arterial blood pressure (MAP).

Reflex. Effects of drugs on reflex increase in R-R interval (R-R), apnoea duration (apnoea), reflex increase in renal nerve activity (RNA) and reflex increase in mean arterial blood pressure (MAP).

0 = No change.

(↑) = Significant increase more than 15 min after drug administration.

↑ = Significant increase at only one time interval, within 15 min of drug administration.

↑↑ = Significant increase at more than one time interval, within 15 min of drug. administration.

(↓) = Significant decrease more than 15 min after drug administration.

↓ = Significant decrease at only one time interval, within 15 min of drug administration.

↓↓ = Significant increase at more than one time interval, within 15 min of drug. administration.

Table 3.13 Summary of results; spontaneously breathing, normoxic rabbit.

Resting.

Drug	R-R	PNA rate	RNA	MAP
Buspirone i.c.	↑↑	↑↑	↓	↓
Buspirone i.c./WAY-100635 i.v.	0	0	0	0
(+)8-OH-DPAT i.c.	↑↑	↑↑	↓	0
(+)8-OH-DPAT i.c./WAY100635 i.v.	0	0	0	0
(+)8-OH-DPAT i.c./GR-127935 i.v.	↑↑	↑↑	↑	0
nn-DP-5-CT i.c.	↑↑	↑↑	↓	0
Sumatriptan i.c./WAY i.v.	0	↑	↑↑	↑
WAY-100635 i.c.	0	0	0	0
WAY-100635 i.v.	0	0	0	0
(-)pindolol i.c.	↑	↓	↓	0
GR-127935 i.c.	(↑)	0	0	0
GR-127935 i.v.	0	0	0	0
Sulpiride i.c.	↓	0	(↑)	↑↑
Mesulergine i.c.	0	0	0	0
Granisetron i.c.	0	0	(↑)	0
Granisetron i.v.	0	0	0	0

Reflex.

Drug	R-R	Apnoea	RNA	MAP
Buspirone i.c.	↑↑	0	0	↓
Buspirone i.c./WAY-100635 i.v.	0	0	0	0
(+)8-OH-DPAT i.c.	↓↓	↓↓	↓↓	↓↓
(+)8-OH-DPAT i.c./WAY100635 i.v.	↓↓	0	0	↓
(+)8-OH-DPAT i.c./GR-127935 i.v.	0	↓↓	0	↓↓
nn-DP-5-CT i.c.	0	0	(↓)	↓↓
Sumatriptan i.c./WAY-100635 i.v.	↓↓	0	0	↓↓
WAY-100635 i.c.	↓↓	0	0	↓
WAY-100635 i.v.	0	0	0	0
(-)pindolol i.c.	↓↓	↓↓	↓↓	↓↓
GR-127935 i.c.	(↑)	0	0	(↑)
GR-127935 i.v.	0	0	0	0
Sulpiride i.c.	0	(↓)	0	(↑)
Mesulergine i.c.	0	0	0	0
Granisetron i.c.	↓↓	↓	0	0
Granisetron i.v.	0	0	0	0

Summary of results.

Effects of 5-HT_{1A} Receptor Agonists.

Administration (i.c.) of the 5-HT_{1A} receptor agonists, buspirone, (+)8-OH-DPAT and nn-DP-5-CT all caused significant increases in baseline R-R interval and phrenic nerve burst rate associated with reduced baseline renal nerve activity. Buspirone increased the reflex increase in R-R interval and reduced the reflex increase in MAP. nn-DP-5-CT had no effect on the reflex increase in R-R interval and apnoea, but reduced the reflex increase in MAP and RNA, and (+)8-OH-DPAT reduced the reflex increase in R-R interval, apnoea duration, RNA and MAP.

Effects of 5-HT_{1A} Receptor Antagonists.

Administration of WAY-100635 (i.c.) had no significant effect on the baseline variables. In contrast, (-)Pindolol (i.c.) significantly increased baseline R-R interval and reduced phrenic burst rate and resting RNA. Both WAY-100635 and (-)pindolol inhibited the reflex increase in R-R interval and MAP, in addition, (-)pindolol also inhibited the apnoea duration and reflex increase in RNA.

Effects of 5-HT_{1A} Receptor Agonists Following Pretreatment with a 5-HT_{1A} Receptor Antagonist.

The increase in baseline R-R interval and phrenic burst rate and reduction in baseline RNA caused by buspirone and (+)8-OH-DPAT (i.c.) were abolished by pretreatment with WAY-100635 (i.v.). The potentiation of the reflex increase in R-R interval and inhibition of the reflex increase in MAP caused by buspirone and inhibition of the apnoea duration and reflex increase in RNA caused by (+)8-OH-DPAT were also abolished by WAY-100635 pretreatment. WAY-100635 pretreatment did not alter the inhibition of the reflex increase in R-R interval and reflex change in MAP caused by (+)8-OH-DPAT.

Effects of 5-HT_{1D} Receptor Agonists.

Administration of sumatriptan (i.c.) in the presence of WAY-100635 (i.v.) to block the effects of this drug as a 5-HT_{1A} receptor agonist, caused a significant increase in phrenic burst rate, resting RNA and MAP. Both the reflex increase in R-R interval and MAP were inhibited.

Effects of a 5-HT_{1D} Receptor Antagonist, GR-127935.

Administration of GR-127935 (i.c.) caused a significant increase in resting R-R interval and potentiated the reflex increase in R-R interval and MAP. When (+)8-OH-DPAT was administered (i.c.) in the presence of GR-127935 (i.v.), the effects of (+)8-OH-DPAT on the resting variables were unchanged, but the inhibition of the reflex increase in R-R interval and RNA caused by (+)8-OH-DPAT alone were abolished.

Effects of a 5-HT_3 Receptor Antagonist, granisetron.

Administration of granisetron (i.c.) caused a significant increase in resting RNA, but did not alter the other resting variables. The reflex increase in R-R interval and apnoea were inhibited by granisetron (i.c.). Granisetron (i.v.) had no effect on either baseline or reflex effects.

Experiments on ventilated rabbits, stimulated with smoke.

Passing a bolus of nicotine-free herbal cigarette smoke (15 - 45 ml) through the upper airways of a **ventilated**, anaesthetised rabbit (n = 10) also triggers the "diving response" described previously. The response was qualitatively similar to that evoked in the spontaneously breathing rabbit, but the increase in R-R interval was significantly reduced to 46 ± 19 ms. The apnoea duration (26 ± 6 s) and increase in mean arterial pressure (19 ± 4 mmHg) were not changed significantly.

Saline (i.c.).

Resting values.

Intracisternal injection of saline (20 μl; n=5; Figure 3.20 and Table 3.14) had little effect on the resting R-R interval, mean arterial blood pressure, renal nerve activity and the rate of phrenic bursts.

Reflex responses to smoke.

Saline did not cause any change to the smoke evoked increase in R-R interval, mean arterial blood pressure, apnoea duration or renal nerve activity (Figure 3.21 and Table 3.15).

Buspirone (i.c.).

Resting values.

Administration of buspirone (i.c.; 200 μg kg^{-1}; n=5; Figure 3.20 and Table 3.14) caused a significant increase in resting R-R interval after 5 min of 36 ± 18 ms. After 15 min this was associated with an decrease in mean arterial pressure of -8 ± 2 mmHg. There were no significant changes in resting phrenic burst rate or renal nerve activity.

Reflex responses to smoke.

Only at 5 min after administration of buspirone was there a significant increase in the smoke evoked reflex increase in R-R interval of 71 ± 56 ms (Figure 3.21 and Table 3.15). This was associated with a significant reduction in the smoke evoked increase in renal nerve activity of -21 ± 6 %, which was also significant at 15 and 25 min. The increase in mean arterial blood pressure and the apnoea duration were unaffected.

Experiments on hyperoxic, spontaneously breathing rabbits, stimulated with smoke.

In the experiments on normoxic, spontaneously breathing rabbits, resting Pa_{O_2} was 106 ± 8 mmHg, which was reduced to 68 ± 11 mmHg after 15 s of apnoea. To investigate the "diving response" in the absence of hypoxic stimulation of the chemoreceptors, resting Pa_{O_2} was increased to 189 ± 8 mmHg, falling to 141 ± 10 mmHg after 15 s of apnoea. Passing a bolus of nicotine-free herbal cigarette smoke (15 - 45 ml) through the upper airways of a **hyperoxic**, spontaneously breathing rabbit (n = 8) triggered a "diving response" that was not significantly different from that evoked in normoxic rabbits.

Saline (i.c.).

Resting values.

Intracisternal (i.c.) injection of saline (20 µl; n=4; Figure 3.22 and Table 3.16) had little effect on the resting R-R interval, mean arterial blood pressure, renal nerve activity and the rate of phrenic bursts.

Reflex responses to smoke.

Saline did not change the smoke evoked increase in R-R interval, mean arterial blood pressure, apnoea duration or renal nerve activity (Figure 3.23 and Table 3.17).

Buspirone (i.c.).

Resting values.

Administration of buspirone (i.c.; 200 $\mu g\ kg^{-1}$; n=4; Figure 3.22 and Table 3.16) caused a significant increase in resting R-R interval after 5 min by 54 ± 16 ms, a significantly greater increase than that seen in normoxic rabbits (23 ± 8 ms). This was associated with a significant decrease in resting mean arterial blood pressure of -12 ± 4 mmHg and a significant increase in phrenic burst rate of 17 ± 13 bursts min^{-1}. There was no significant change in resting renal nerve activity. The effects of buspirone on resting mean arterial pressure, phrenic burst rate and RNA were not significantly different from those in normoxic rabbits.

Reflex responses to smoke.

15 min after administration of buspirone there was a significant potentiation of the smoke evoked increase in R-R interval of 103 ± 24 ms, which persisted for 35 min. This was not associated with a significant change in any of the other variables (Figure 3.23 and Table 3.17). None of the variables differed significantly from those in normoxic rabbits.

Reflex responses to Phenylbiguanide given into the right atrium of the anaesthetised rabbit.

Administration of phenylbiguanide (PBG; 10 - 25 $\mu g\ kg^{-1}$) into the right atrium caused a reflex increase in R-R interval of 39 ± 10 ms. This was associated with a sympathoinhibition, a hypotension (-20 ± 3 mmHg) and an increase in respiratory rate which could become a fused inspiratory tetanus. The R-R interval and mean arterial pressure returned to baseline rapidly after the PBG challenge, usually within 60 s of the stimulus being given. Renal nerve activity and phrenic rate often took longer to return to baseline, but had always recovered 5 min after the challenge. A typical response to PBG is illustrated in Figure 3.24. Experiments were only performed when a stable reflex with an increase in R-R interval of at least 25 ms could be elicited.

Saline (i.c.).

Resting values.

Intracisternal injection saline (20 μl; n=5) had little effect on the resting R-R interval or mean arterial blood pressure (Table 5.56 in Appendix 5.5).

Reflex responses to PBG.

Saline did not change the PBG evoked increase in R-R interval or reduction in mean arterial blood pressure (Figure 3.25 and Table 3.18).

Buspirone (i.c.).

Resting values.

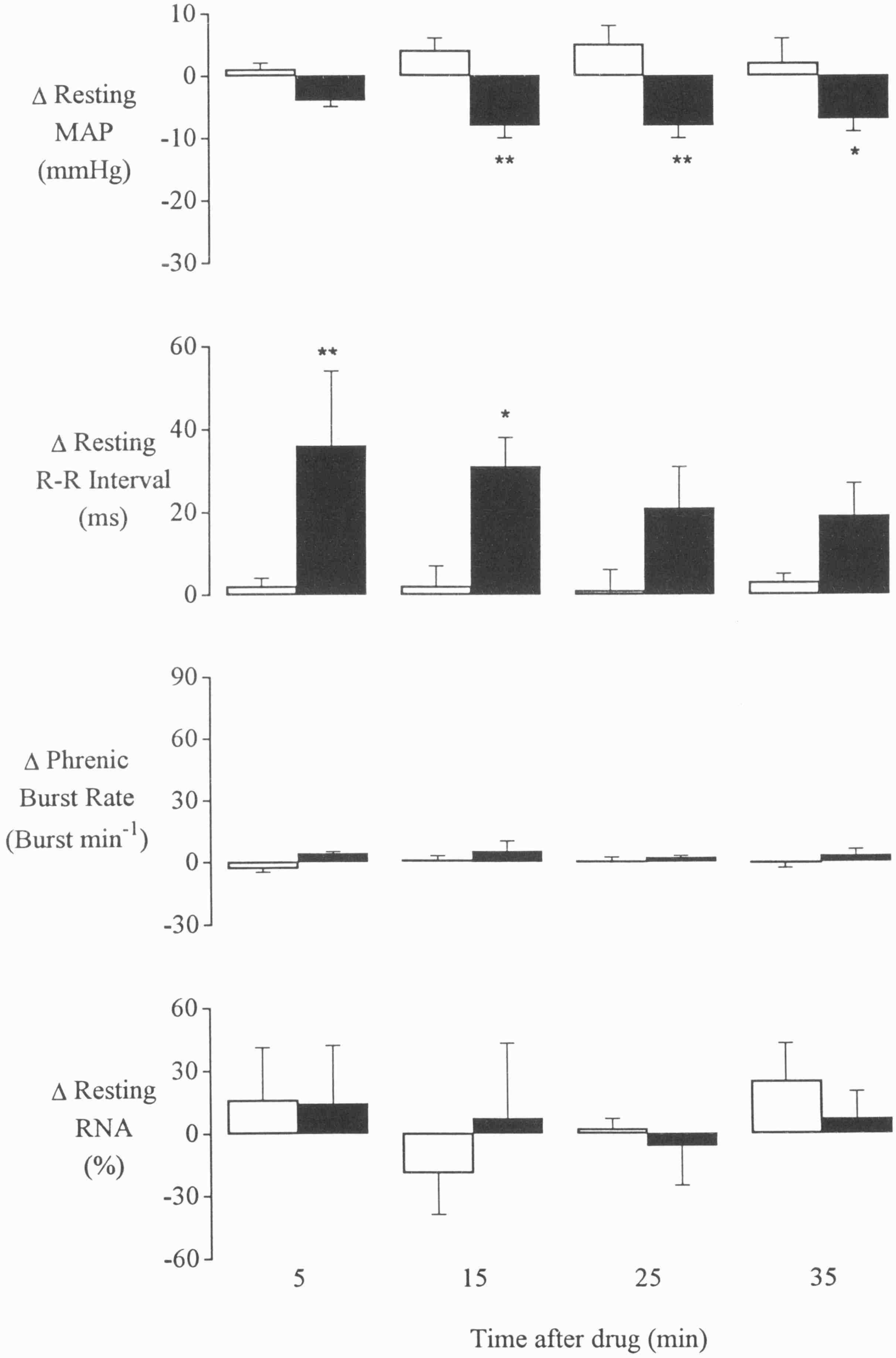
Administration of buspirone (i.c.; 200 μg kg^{-1}; n=5) caused a significant increase in the resting R-R interval of 17 ± 4 ms after 5 min. This was not associated with a significant change in baseline mean arterial blood pressure (Table 5.57 in Appendix 5.5).

Reflex responses to PBG.

15 min after administration of buspirone there was a significant potentiation of the PBG evoked increase in R-R interval of 60 ± 17 ms. This was not associated with a change in the reflex decrease in mean arterial blood pressure (Figure 3.25 and Table 3.18).

Figure 3.20 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, artificially ventilated rabbits: histograms showing changes (Δ) in resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic nerve burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min after intracisternal (i.c.) injections of buspirone (200 μg kg^{-1}; ■ ; n=5) and saline (20 μl; □ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e.mean. Changes caused by buspirone have been compared with those caused by saline using ANOVA and least significant difference test.

* p<0.05; ** p<0.01.



10
0
-10
-20
-30
Δ Resting
MAP
(mmHg)
**
**
*
60
40
20
0
Δ Resting
R-R Interval
(ms)
**
*
90
60
30
0
-30
Δ Phrenic
Burst Rate
(Burst min^{-1})
60
30
0
-30
-60
Δ Resting
RNA
(%)
5
15
25
35
Time after drug (min)

Figure 3.21 Anaesthetised, atenolol (i.v. 1 mg kg^{-1}) pretreated, normoxic, artificially ventilated rabbits: histograms showing changes (Δ) in the reflex increases in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min after intracisternal (i.c.) injections of buspirone (200 $\mu g\ kg^{-1}$; ■ ; n=5) and saline (20 μl; □ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by buspirone have been compared with those caused by saline using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

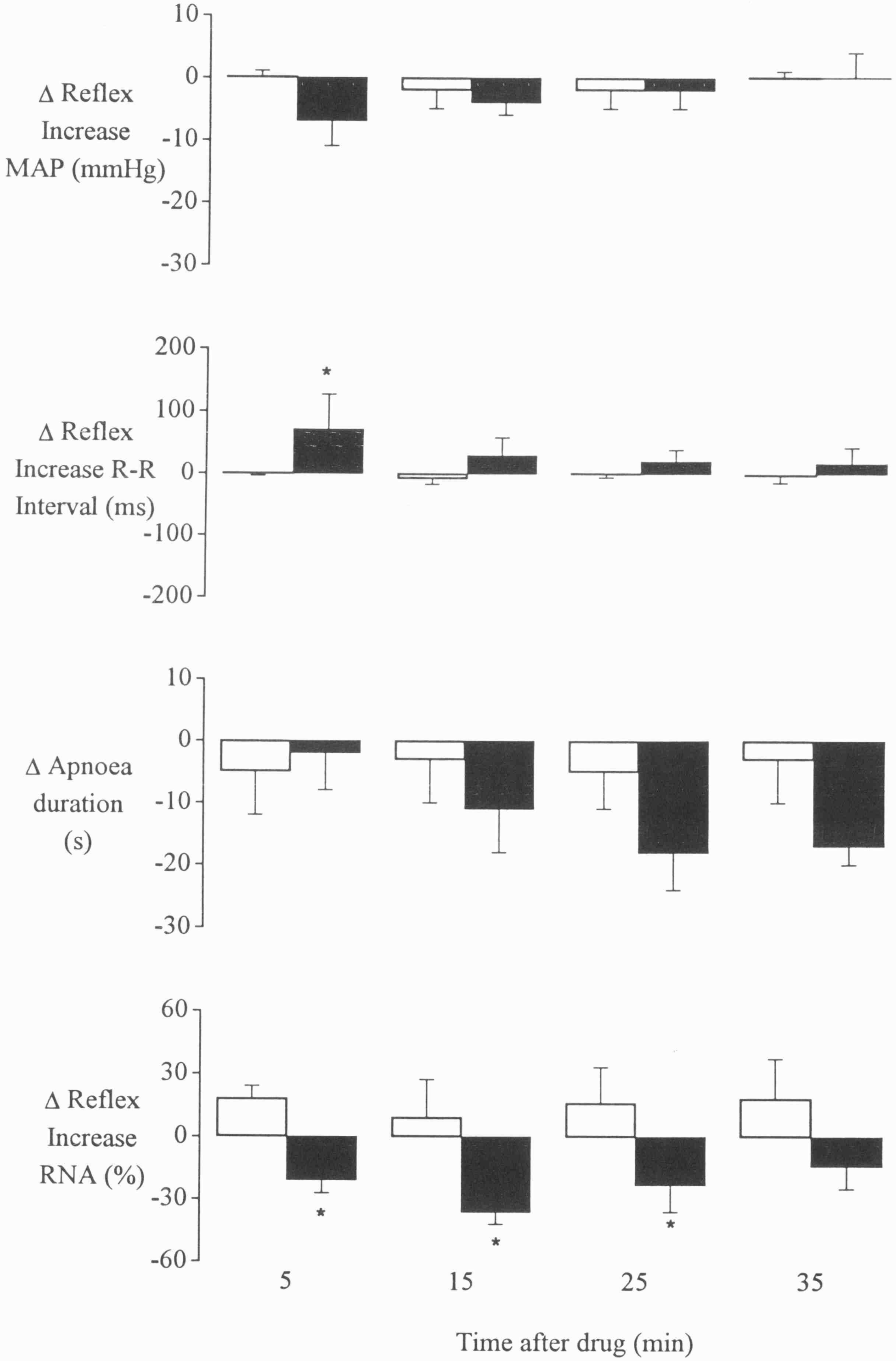
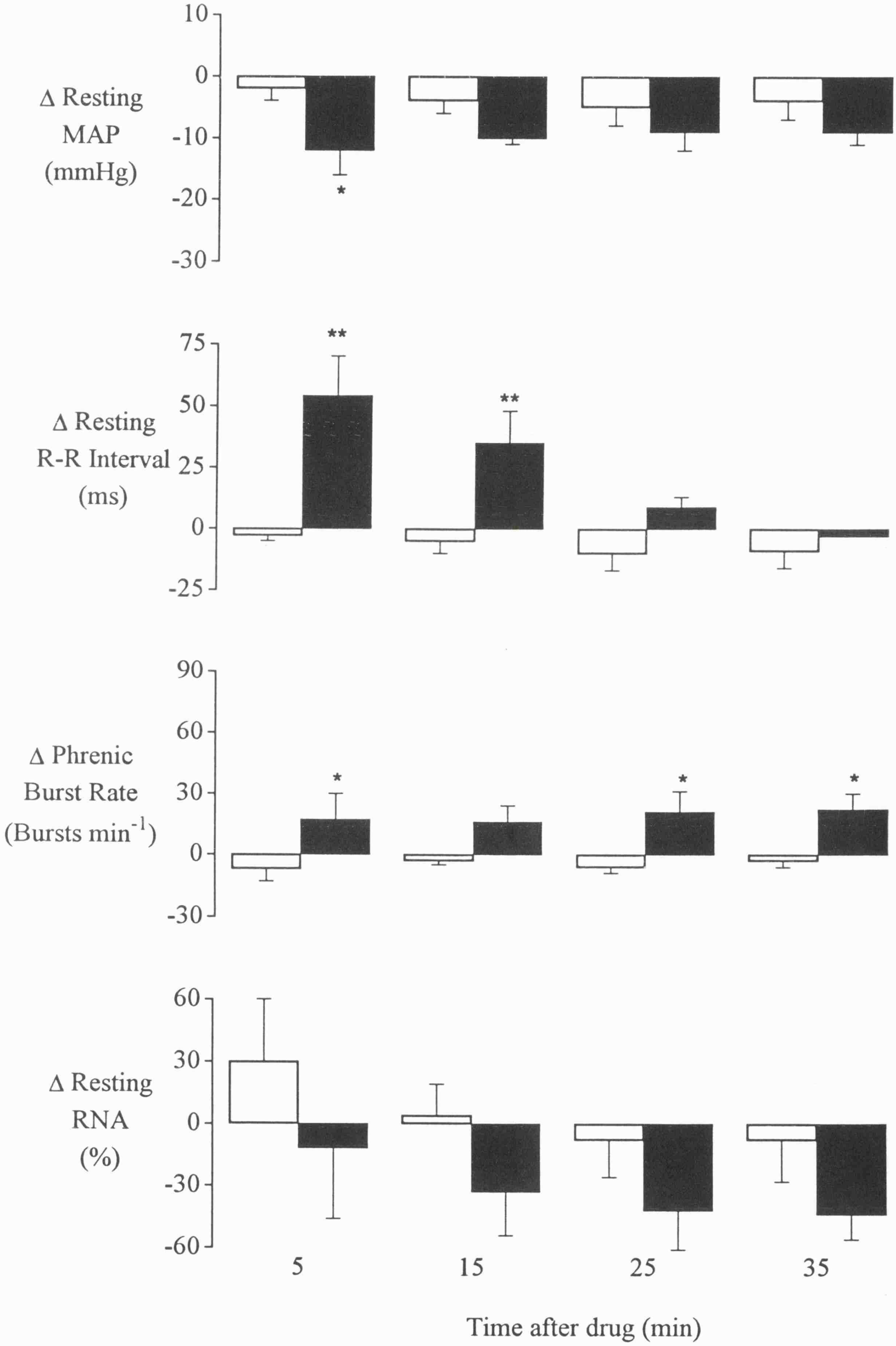

10
0
-10
-20
-30
Δ Reflex
Increase
MAP (mmHg)
200
100
0
-100
-200
*
Δ Reflex
Increase R-R
Interval (ms)
10
0
-10
-20
-30
Δ Apnoea
duration
(s)
60
30
0
-30
-60
*
*
*
Δ Reflex
Increase
RNA (%)
5
15
25
35
Time after drug (min)

Figure 3.22 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, hyperoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic nerve burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min after intracisternal (i.c.) injections of buspirone (200 μg kg^{-1}; ■ ; n=5) and saline (20 μl; □ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e.mean. Changes caused by buspirone have been compared with those caused by saline using ANOVA and least significant difference test.

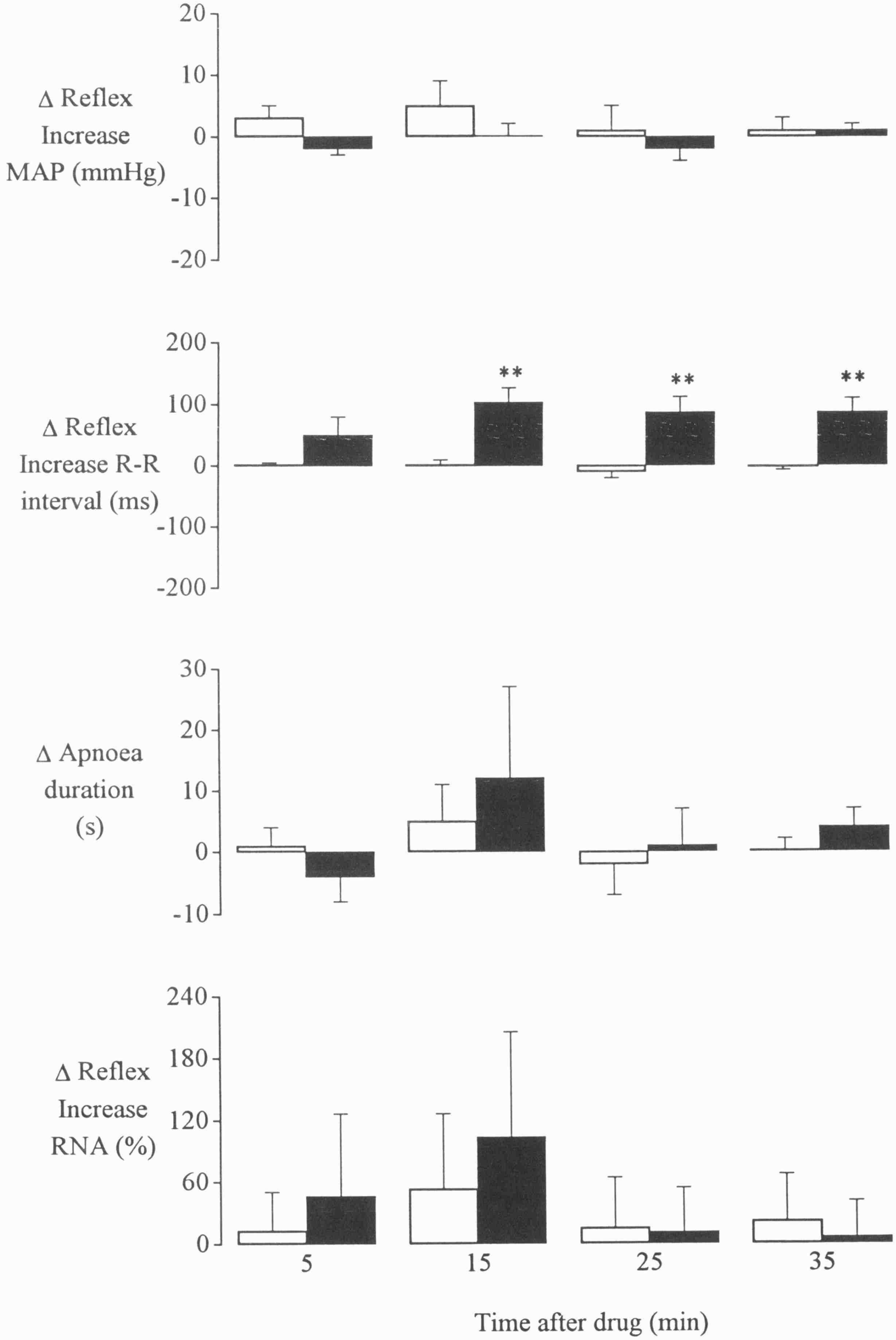
* $p<0.05$; ** $p<0.01$.



10
0
-10
-20
-30
Δ Resting
MAP
(mmHg)
*
75
50
25
0
-25
Δ Resting
R-R Interval
(ms)
**
**
90
60
30
0
-30
Δ Phrenic
Burst Rate
(Bursts min-1)
*
*
*
60
30
0
-30
-60
Δ Resting
RNA
(%)
5
15
25
35
Time after drug (min)

Figure 3.23 Anaesthetised, atenolol (i.v. 1 mg kg^{-1}) pretreated, hyperoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in the reflex increases in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min after intracisternal (i.c.) injections of buspirone (200 μg kg^{-1}; ■ ; n=5) and saline (20 μl; □ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by buspirone have been compared with those caused by saline using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.



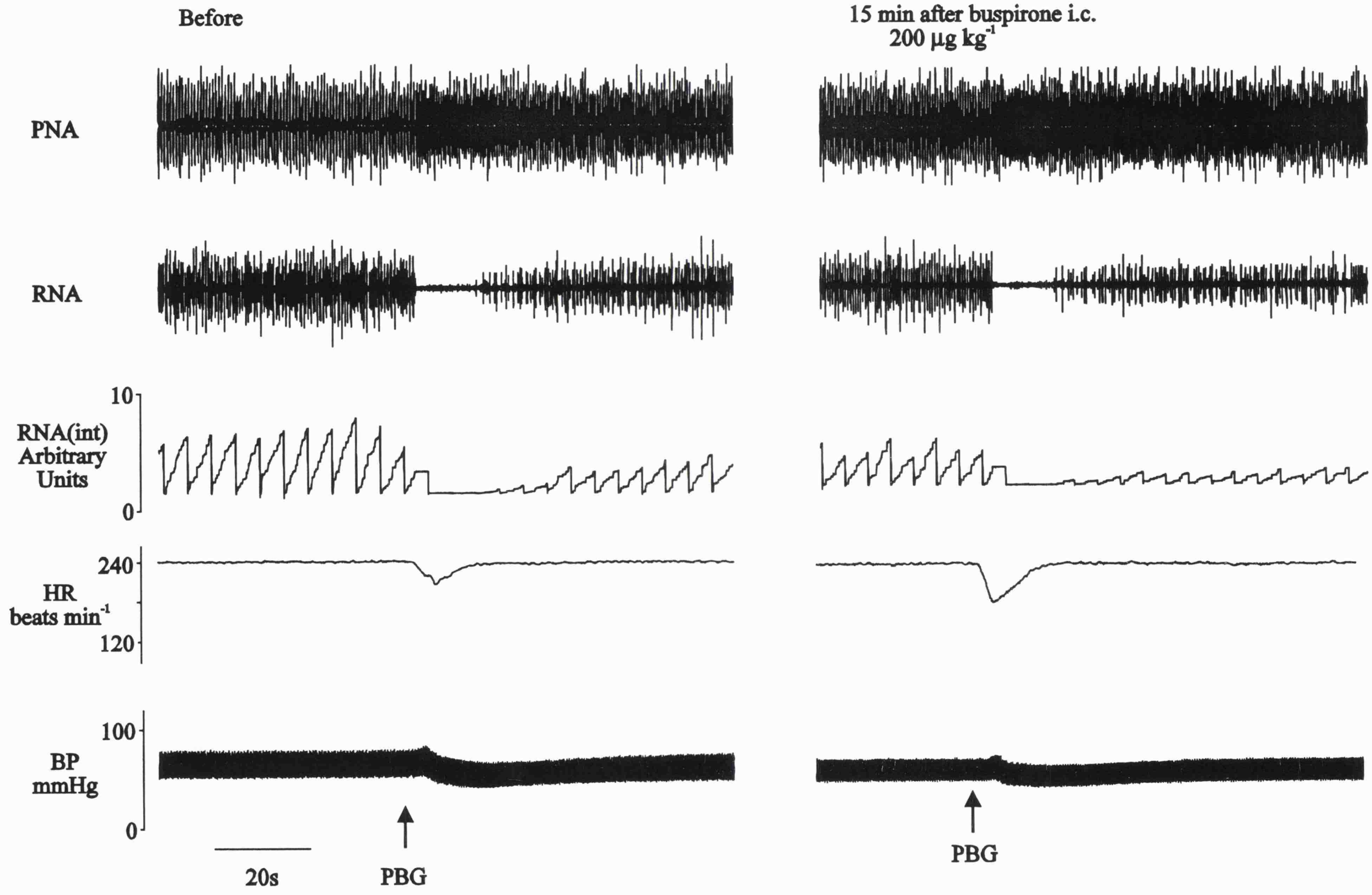
Δ Reflex Increase MAP (mmHg)
20
10
0
-10
-20
Δ Reflex Increase R-R interval (ms)
200
100
0
-100
-200
**
**
**
Δ Apnoea duration (s)
30
20
10
0
-10
Δ Reflex Increase RNA (%)
240
180
120
60
0
5
15
25
35
Time after drug (min)

Figure 3.24

Trace shows the response to phenylbiguanide (PBG; 10 - 30 μg kg^{-1}) delivered to the right atrium, in an atenolol (1 mg kg^{-1}; i.v.) pretreated rabbit, 5 min before and 15 min after administration of 200 μg kg^{-1} buspirone i.c.

From the top, the traces illustrate phrenic nerve activity (PNA), renal nerve activity (RNA), renal nerve activity integrated with a 5 s time constant (RNA(int)), heart rate (HR) and blood pressure (BP). Each trace is 120 s in duration.

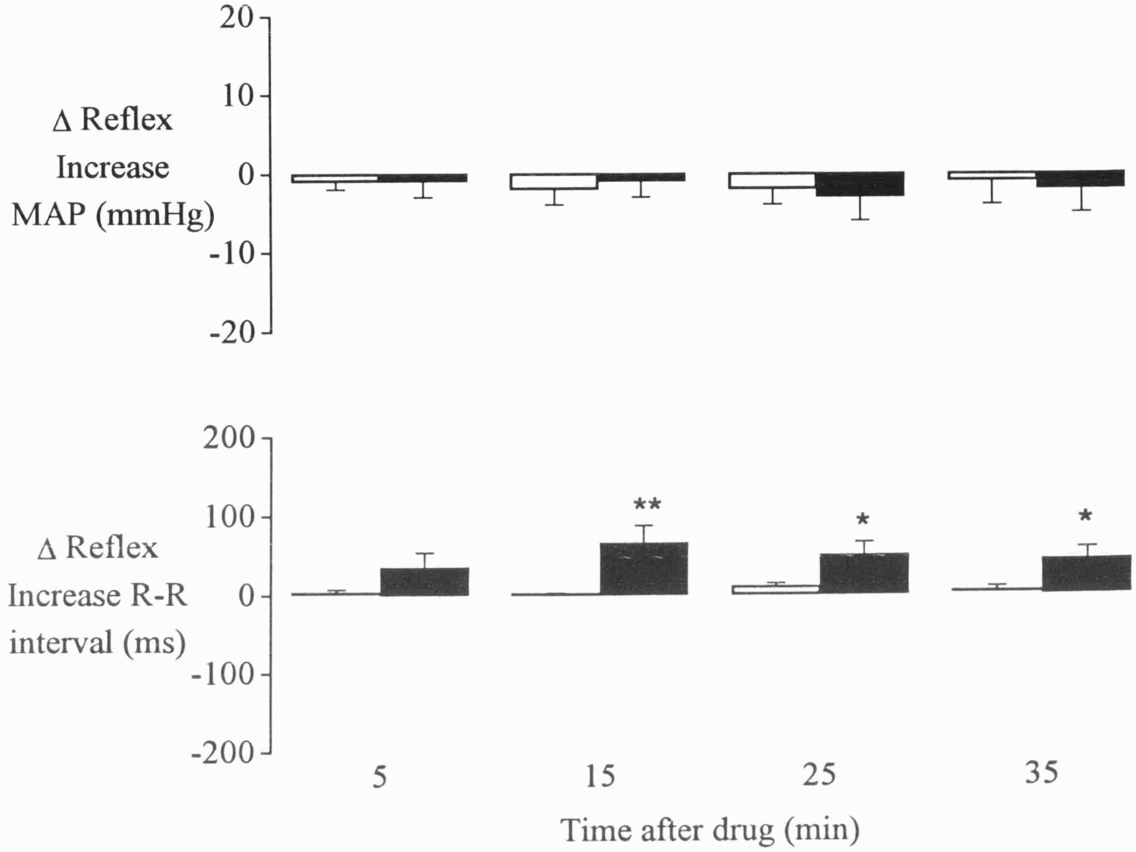
A bolus of phenylbiguanide was injected into the right atrium at the point marked by an arrow and the letters PBG.



Before
15 min after buspirone i.c.
200 μg kg-1
PNA
RNA
RNA(int)
Arbitrary
Units
10
0
HR
beats min-1
240
120
BP
mmHg
100
0
20s
PBG
PBG

Figure 3.25 Anaesthetised, atenolol (i.v. 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rabbits: histograms showing changes (Δ) in the reflex decreases in mean arterial blood pressure (MAP; mmHg) and R-R interval (ms) elicited by stimulating the right atrium with phenylbiguanide 5 min after intracisternal (i.c.) injections of buspirone (200 μg kg^{-1}; ■ ; n=5) and saline (20 μl; □ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by buspirone have been compared with those caused by saline using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.



20
10
Δ Reflex
Increase
MAP (mmHg)
0
-10
-20
200
100
**
*
*
Δ Reflex
Increase R-R
interval (ms)
0
-100
-200
5
15
25
35
Time after drug (min)

Table 3.14 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, artificially ventilated rabbits: showing the absolute values (mean ± s.e. mean) of resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min before and 5 min after administration of saline (20 μl i.c.; n=5) or buspirone (200 μg kg^{-1} i.c.; n=5) and thereafter at 10 min intervals over 35 min. The changes (Δ; mean ± s.e. mean) in resting values of the variables are given in parentheses, calculated from the values at 5 min before administration of the test substances. Time matched values of the drug versus vehicle have been compared using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.14

Saline 20 μl i.c. n=5.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	55 ± 3	56 ± 2 **(1±1)**	59 ± 2 **(4±2)**	59 ± 2 **(5±3)**	57 ± 2 **(2±4)**
R-R interval (ms)	265 ± 5	267 ± 5 **(2±2)**	267 ± 8 **(2±5)**	266 ± 8 **(1±5)**	268 ± 7 **(3±2)**
Phrenic burst rate(bursts min^{-1})	53 ± 3	50 ± 3 **(-3±2)**	54 ± 3 **(1±2)**	53 ± 3 **(0±2)**	53 ± 3 **(-1±2)**
RNA (%)	100	116 ± 25 **(16±25)**	82 ± 20 **(-19±20)**	101 ± 5 **(2±5)**	125 ± 18 **(25±18)**

Buspirone 200 μg kg^{-1} i.c. n=4.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	60 ± 5	56 ± 5 **(-4±1)**	52 ± 3 **(-8±2**)**	52 ± 3 **(-8±2**)**	53 ± 4 **(-7±2*)**
R-R interval (ms)	258 ± 5	294 ± 18* **(36±18**)**	289 ± 7 **(31±7*)**	279 ± 8 **(21±10)**	276 ± 7 **(19±8)**
Phrenic burst rate(bursts min^{-1})	59 ± 5	63 ± 4* **(4±1)**	63 ± 1 **(5±5)**	60 ± 5 **(2±1)**	61 ± 5 **(3±3)**
RNA (%)	100	114 ± 28 **(14±28)**	107 ± 36 **(7±36)**	94 ± 19 **(-6±19)**	107 ± 13 **(7±13)**

Table 3.15 Anaesthetised, atenolol (i.v.; 1 mg kg) pretreated, normoxic, artificially ventilated rabbits: showing the absolute reflex changes (mean ± s.e. mean) in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min before and 5 min after administration of saline (20 μl i.c.; n=5) or buspirone (200 μg kg^{-1} i.c.; n=5) and thereafter at 10 min intervals over 35 min. Changes (Δ; mean ± s.e. mean) in the reflex response of the variables from preinjection (-5 min) values are given. Time matched comparison of drug versus vehicle have been made using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.15

Saline 20 µl i.c. n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	19 ± 5	19 ± 3 **(0±1)**	17 ± 2 **(-2±3)**	17 ± 4 **(-2±3)**	19 ± 4 **(0±1)**
Increase R-R interval (ms)	41 ± 18	40 ± 15 **(-1±3)**	33 ± 8 **(-8±10)**	41 ± 12 **(-1±6)**	37 ± 8 **(-5±10)**
Apnoea duration (s)	29 ± 5	23 ± 3 **(-5±7)**	26 ± 4 **(-3±7)**	23 ± 5 **(-5±6)**	25 ± 6 **(-3±7)**
RNA (%)	100	118 ± 6 **(18±6)**	109 ± 18 **(9±18)**	116 ± 17 **(16±17)**	118 ± 19 **(18±19)**

Buspirone 200 µg kg^{-1} i.c. n=4.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	20 ± 3	14 ± 3 **(-7±4)**	16 ± 2 **(-4±2)**	18 ± 2 **(-2±3)**	20 ± 5 **(0±4)**
Increase R-R interval (ms)	51 ± 19	122 ± 58* **(71±56*)**	80 ± 28 **(29±29)**	70 ± 13 **(19±19)**	66 ± 22 **(16±26)**
Apnoea duration (s)	24 ± 7	22 ± 10 **(-2±6)**	13 ± 7 **(-11±7)**	7 ± 4* **(-18±6)**	7 ± 4* **(-17±3)**
RNA (%)	100	80 ± 6* **(-21±6*)**	64 ± 6* **(-36±6*)**	78 ± 13* **(-23±13)**	86 ± 11 **(-14±11)**

Table 3.16 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, hyperoxic, spontaneously breathing rabbits: showing the absolute values (mean ± s.e. mean) of resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min before and 5 min after administration of saline (20 μl i.c.; n=5) or buspirone (200 μg kg^{-1} i.c.; n=5) and thereafter at 10 min intervals over 35 min. The changes (Δ; mean ± s.e. mean) in resting values of the variables are given in parentheses, calculated from the values at 5 min before administration of the test substances. Time matched values of the drug versus vehicle have been compared using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.16

Saline 20 μl i.c. n=4.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	58 ± 3	55 ± 3 **(-2±2)**	53 ± 3 **(-4±2)**	51 ± 4 **(-5±3)**	51 ± 5 **(-4±3)**
R-R interval (ms)	266 ± 5	263 ± 6 **(-3±2)**	261 ± 7 **(-5±5)**	256 ± 7 **(-10±7)**	257 ± 7 **(-9±7)**
Phrenic burst rate(bursts min^{-1})	69 ± 5	62 ± 2 **(-7±6)**	66 ± 6 **(-3±2)**	63 ± 4 **(-6±3)**	66 ± 5 **(-3±3)**
RNA (%)	100	105 ± 7 **(30±30)**	104 ± 15 **(4±15)**	89 ± 19 **(-8±18)**	92 ± 20 **(-8±20)**

Buspirone 200 μg kg^{-1} i.c. n=4.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	58 ± 6	52 ± 6 **(-12±4*)**	53 ± 5 **(-10±1)**	56 ± 6 **(-9±3)**	57 ± 6 **(-9±2)**
R-R interval (ms)	260 ± 17	314 ± 23* **(54±16**)**	295 ± 29 **(35±13**)**	269 ± 21 **(9±4)**	258 ± 18 **(-3±3)**
Phrenic burst rate(bursts min^{-1})	60 ± 8	77 ± 8 **(17±13*)**	76 ± 5 **(16±8)**	81 ± 7 **(21±10*)**	82 ± 11 **(22±8*)**
RNA (%)	100	88 ± 34 **(-12±34)**	67 ± 21 **(-33±21)**	58 ± 19 **(-42±19)**	12 ± 56 **(-44±12)**

Table 3.17 Anaesthetised, atenolol (i.v.; 1 mg kg) pretreated, hyperoxic, spontaneously breathing rabbits: showing the absolute reflex changes (mean ± s.e. mean) in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min before and 5 min after administration of saline (20 μl i.c.; n=5) or buspirone (200 μg kg^{-1} i.c.; n=5) and thereafter at 10 min intervals over 35 min. Changes (Δ; mean ± s.e. mean) in the reflex response of the variables from preinjection (-5 min) values are given. Time matched comparison of drug versus vehicle have been made using ANOVA and least significant difference test.
* $p<0.05$; ** $p<0.01$.

Table 3.17

Saline 20 μl i.c. n=4.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	22 ± 4	25 ± 2 **(3±2)**	27 ± 1 **(5±4)**	23 ± 2 **(1±4)**	23 ± 3 **(1±2)**
Increase R-R interval (ms)	72 ± 20	73 ± 18 **(0±4)**	73 ± 11 **(0±9)**	61 ± 19 **(-11±10)**	68 ± 21 **(-5±3)**
Apnoea duration (s)	16 ± 4	16 ± 1 **(1±3)**	21 ± 5 **(5±6)**	14 ± 6 **(-2±5)**	16 ± 5 **(0±2)**
RNA (%)	100	113 ± 37 **(13±27)**	153 ± 73 **(53±73)**	115 ± 49 **(15±49)**	122 ± 45 **(22±45)**

Buspirone 200 μg kg^{-1} i.c. n=4.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	19 ± 2	17 ± 3* **(-2±1)**	19 ± 2* **(0±2)**	17 ± 4 **(-2±2)**	20 ± 2 **(1±1)**
Increase R-R interval (ms)	70 ± 8	126 ± 38 **(49±30)**	173±31****(103±24**)**	156 ± 33* **(86±26**)**	155 ± 30* **(86±23**)**
Apnoea duration (s)	18 ± 4	14 ± 1 **(-4±4)**	22 ± 7 **(12±15)**	19 ± 4 **(1±6)**	21 ± 3 **(4±3)**
RNA (%)	100	146 ± 80 **(46±80)**	203 ± 102 **(103±102)**	111 ± 43 **(11±43)**	106 ± 35 **(6±35)**

Table 3.18 Anaesthetised, atenolol (i.v.; 1 mg kg) pretreated, normoxic, spontaneously breathing rabbits: showing the absolute reflex changes (mean ± s.e. mean) in mean arterial blood pressure (MAP; mmHg) and R-R interval (ms) elicited by stimulating the right atrium with phenylbiguanide 5 min before and 5 min after administration of saline (20 μl i.c.; n=5) or buspirone (200 μg kg^{-1} i.c.; n=5) and thereafter at 10 min intervals over 35 min. Changes (Δ; mean ± s.e. mean) in the reflex response of the variables from preinjection (-5 min) values are given. Time matched comparison of drug versus vehicle have been made using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.18

Saline 20 µl i.c. n=5.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	-23 ± 5	-22 ± 5 **(-1±2)**	-21 ± 5 **(-2±2)**	-20 ± 5 **(-3±3)**	-21 ± 5 **(-4±2)**
R-R interval (ms)	37 ± 7	41 ± 7 **(4±3)**	39 ± 6 **(1±1)**	47 ± 7 **(10±4)**	42 ± 9 **(5±4)**

Buspirone 200 µg kg^{-1} i.c. n=5.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	-17 ± 2	-14 ± 2 **(-3±1)**	-13 ± 2 **(-5±1)**	-14 ± 2 **(-4±2)**	-14 ± 2 **(-3±2)**
R-R interval (ms)	40 ± 13	66 ± 22 **(26±15)**	100 ± 27* **(60±17**)**	89 ± 26 **(43±17*)**	83 ± 24 **(37±15*)**

Reflex responses to upper airway stimulation of the rat with smoke.

Passing a bolus of nicotine-free herbal cigarette smoke (5 - 30 ml) through the upper airways of spontaneously breathing, anaesthetised rats (n = 35) evokes a "diving response" that is qualitatively similar to that already described in the rabbit. The expiratory apnoea was shorter in the rats (6 ± 1 s) and there was a smaller increase in R-R interval, with an increase of 15 ± 3 ms at 3 s after stimulation of the upper airways and a peak increase of 38 ± 10 ms from a resting R-R interval of 164 ± 4 ms. Although the absolute size of the R-R increase was smaller in the rat, the increase as a percentage of resting R-R interval was similar between rats and rabbits. The smoke evoked increase in mean arterial pressure was 41 ± 6 mmHg, which was larger than the response in rabbits and was associated with a higher resting mean arterial pressure of 81 ± 6 mmHg. An example of the response to smoke stimulation in the rat is illustrated in Figure 3.26.

Experiments on spontaneously breathing rats, stimulated with smoke.

These results are summarised in Table 3.23.

Effects of vehicles.

Resting values.

Intracisternal (i.c.) injection of saline (20 µl; n=5; Figure 3.28 and Table 3.19) or acidified saline (20 µl; pH 2.0; n=4; Table 5.69 in Appendix 5.5) had little effect on the resting R-R interval, mean arterial blood pressure or phrenic burst rate.

Reflex responses to smoke.

Neither saline (Figure 3.29 and Table 3.20) nor acidified saline (i.c.) (Table 5.80 in Appendix 5.5) caused any change to the smoke evoked reflex increase in R-R interval either at 3 s after stimulation of the upper airways or at the peak of the response, mean arterial blood pressure or apnoea duration.

Effect of 5-HT_{1A} receptor ligands.

Buspirone (i.c.).

Resting values.

Administration of buspirone (i.c.; 200 μg kg^{-1}; n=5; Figure 3.28 and Table 3.19a) caused a significant increase in resting R-R interval after 15 min of 10 ± 6 ms, this was not associated with any significant change in mean arterial blood pressure. Phrenic burst rate was significantly increased after 5 min by 29 ± 9 bursts min^{-1}.

Reflex responses to smoke.

15 min after administration of buspirone there was a significant inhibition of the smoke induced increase in R-R interval of -27 ± 8 ms (Figure 3.29 and Table 3.20a). This was associated with a reduction in the apnoea duration of -5 ± 1 s, but no significant change in mean arterial blood pressure. A trace of one of these experiments is shown in Figure 3.26.

(+)8-OH-DPAT (i.c.).

Resting values.

Administration of (+)8-OH-DPAT caused a significant increase in resting R-R interval of 10 ± 4 ms after 15 min (Figure 3.28 and Table 3.19b). This was not associated with a significant change in phrenic burst rate or mean arterial blood pressure, although both variables were significantly changed during the course of the experiment. Phrenic burst rate was significantly increased after 5 min by 59 ± 5 bursts min^{-1}. Mean arterial blood pressure was significantly reduced by -12 ± 4 mmHg after 35 min.

Reflex responses to smoke.

5 min after administration of (+)8-OH-DPAT there was a significant reduction in the smoke evoked increase in R-R interval by -32 ± 8 ms (Figure 3.29 and Table 3.20b). This was associated with a significant shortening of the apnoea duration by

-6 ± 1 s, but no significant changes to the smoke evoked increase in mean arterial blood pressure. A trace of one of these experiments is shown in Figure 3.27.

WAY-100635 (i.c.).

Resting values.

Administration of WAY-100635 (i.c.; 100 μg kg^{-1}; n=4) did not significantly change resting R-R interval (Table 5.65 in Appendix 5.5). There were significant effects on resting mean arterial blood pressure and phrenic burst rate. MAP was reduced by -17 ± 3 mmHg after 5 min and phrenic burst rate was reduced by -52 ± 15 bursts min^{-1}.

Reflex responses to smoke.

WAY-100635 had no significant effect on the smoke induced reflex increase in R-R 3 s after stimulation of the upper airways, the peak increase in R-R interval or the reflex increase in mean arterial blood pressure. The drug did cause a significant potentiation of the apnoea length of 4 ± 2 s after 15 min (Table 5.76 in Appendix 5.5).

WAY-100802 (i.c.).

Resting values.

Administration of WAY-100802 (i.c.; 50 μg kg^{-1}; n=5) had no significant effect on baseline MAP or R-R interval, but caused a highly significant reduction in respiratory rate by -37 ± 9 breaths $minute^{-1}$ after 5 min (Table 5.66 in Appendix 5.5).

Reflex responses to smoke.

Administration WAY-100802 did not significantly alter the size of the reflex bradycardia or the hypertension, but it did significantly lengthen the apnoea duration by 2 ± 1 s after 5 minutes only (Table 5.77 in Appendix 5.5).

Pretreatment with WAY-100802 (i.c.) before (+)8-OH-DPAT (i.c.).

Resting values.

The effect of (+)8-OH-DPAT (i.c.; 25 $\mu g\ kg^{-1}$; n=5) on resting R-R interval and mean arterial blood pressure was not significantly changed by pretreatment with WAY-100802 (i.c.; 50 $\mu g\ kg^{-1}$) 20 minutes earlier (Figure 3.30 and Table 3.21). The (+)8-OH-DPAT mediated increase in phrenic burst rate was reduced to 9 ± 11 bursts min^{-1} by pretreatment, compared with an increase of 59 ± 5 bursts min^{-1} in the untreated rats.

Reflex responses to smoke.

There were significant differences between the effects of (+)8-OH-DPAT on the reflex responses to smoke in untreated and WAY-100802 pretreated rats (Figure 3.31 and Table 3.22). The inhibition of the smoke evoked increase in R-R interval after 5 min was reduced from -32 ± 8 ms to 3 ± 7 ms by pretreatment. This was associated with a reduction in the shortening of the apnoea duration from -6 ± 1 s to -2 ± 1 s following pretreatment and a change of (+)8-OH-DPAT mediated reduction of the reflex increase in mean arterial blood pressure by -8 ± 5 mmHg to an increase in the response of 7 ± 4 mmHg.

(-)Pindolol (i.c.).

Resting values.

Administration of (-)pindolol (i.c.; 100 $\mu g\ kg^{-1}$; n=4) did not significantly change the resting mean arterial blood pressure or R-R interval (Table 5.70 in Appendix 5.5). The phrenic burst rate was significantly reduced by -54 ± 14 bursts min^{-1} after 5 minutes.

Reflex responses to smoke.

5 min after administration of (-)pindolol there was a significant inhibition of the smoke evoked reflex increase in R-R interval of -21 ± 6 ms. This was associated with a significant reduction in the reflex increase in mean arterial blood pressure of -25 ± 7 mmHg. There was no significant change to the apnoea duration (Table 5.81 in Appendix 5.5).

Granisetron (i.c.).

Resting values.

Administration of granisetron (i.c.; 20 μg kg^{-1}; n=3) caused no significant changes to resting R-R interval, mean arterial blood pressure or phrenic burst rate (Table 5.68 in Appendix 5.5).

Reflex responses to smoke.

The smoke evoked reflex increase in R-R interval and mean arterial blood pressure and the apnoea duration were also unchanged by administration of granisetron i.c. (Table 5.79 in Appendix 5.5).

Preliminary experiments on spontaneously breathing rats, stimulated with phenylbiguanide.

Resting values.

Administration of WAY-100635 (i.c.; 200 μg kg^{-1}; n=4) caused no change to either resting mean arterial blood pressure or R-R interval (Table 5.83 in Appendix 5.5).

Reflex responses to PBG.

WAY-100635 did not alter the size of the pulmonary C-fibre reflex evoked increase in R-R interval or reduction in mean arterial blood pressure. A trace of one of these experiments is illustrated in Figure 3.32 (Table 5.84 in Appendix 5.5).

Figure 3.26

Trace shows the response to smoke delivered to the upper airways, in an atenolol ($1\ mg\ kg^{-1}$; i.v.) pretreated rat, 5 min before and 15 min after administration of 200 $\mu g\ kg^{-1}$ buspirone i.c.

From the top, the traces illustrate phrenic nerve activity (PNA), heart rate (HR) and blood pressure (BP). Each trace is 120 s in duration.

A bolus of smoke was passed through the upper airways at the point marked by an arrow and the letter S.

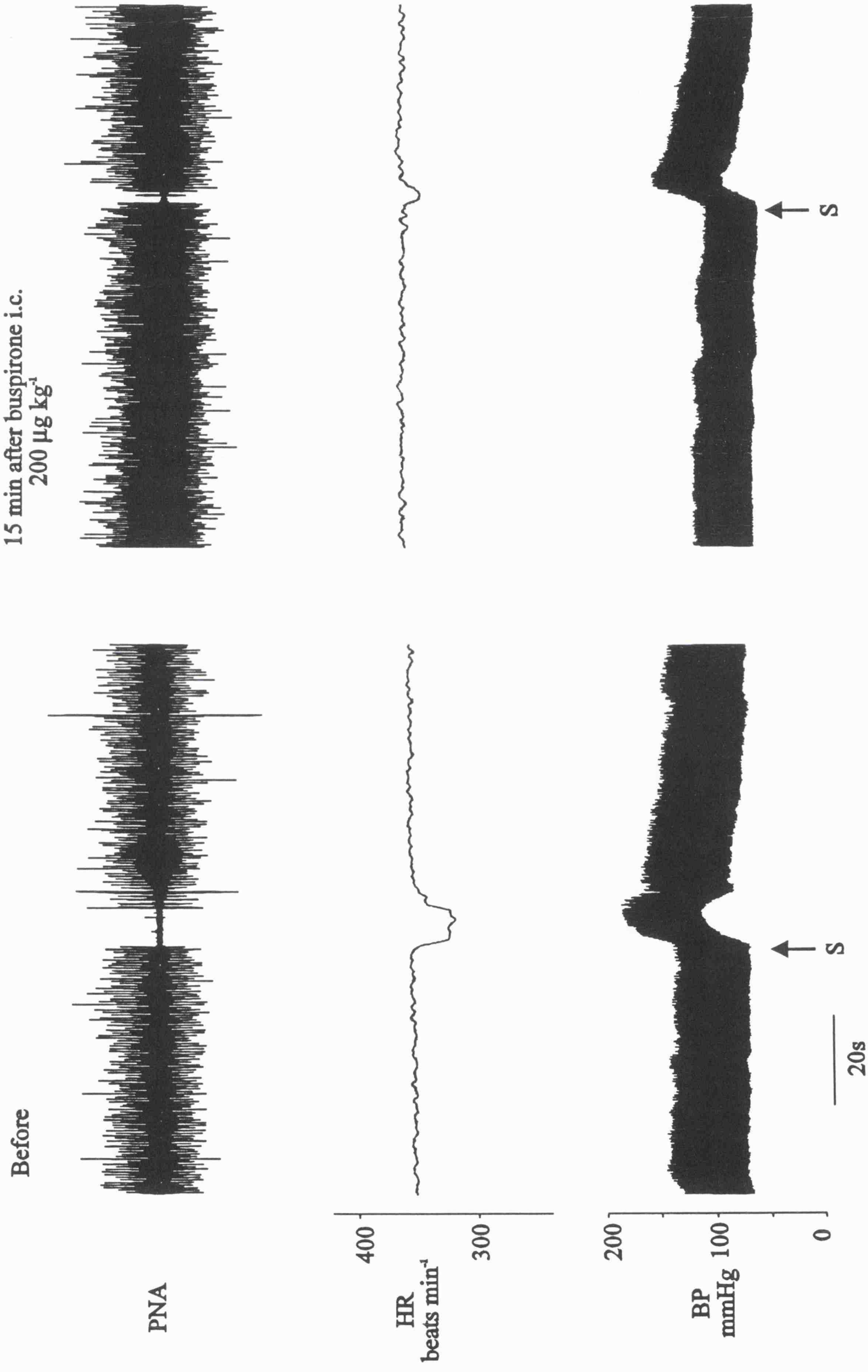
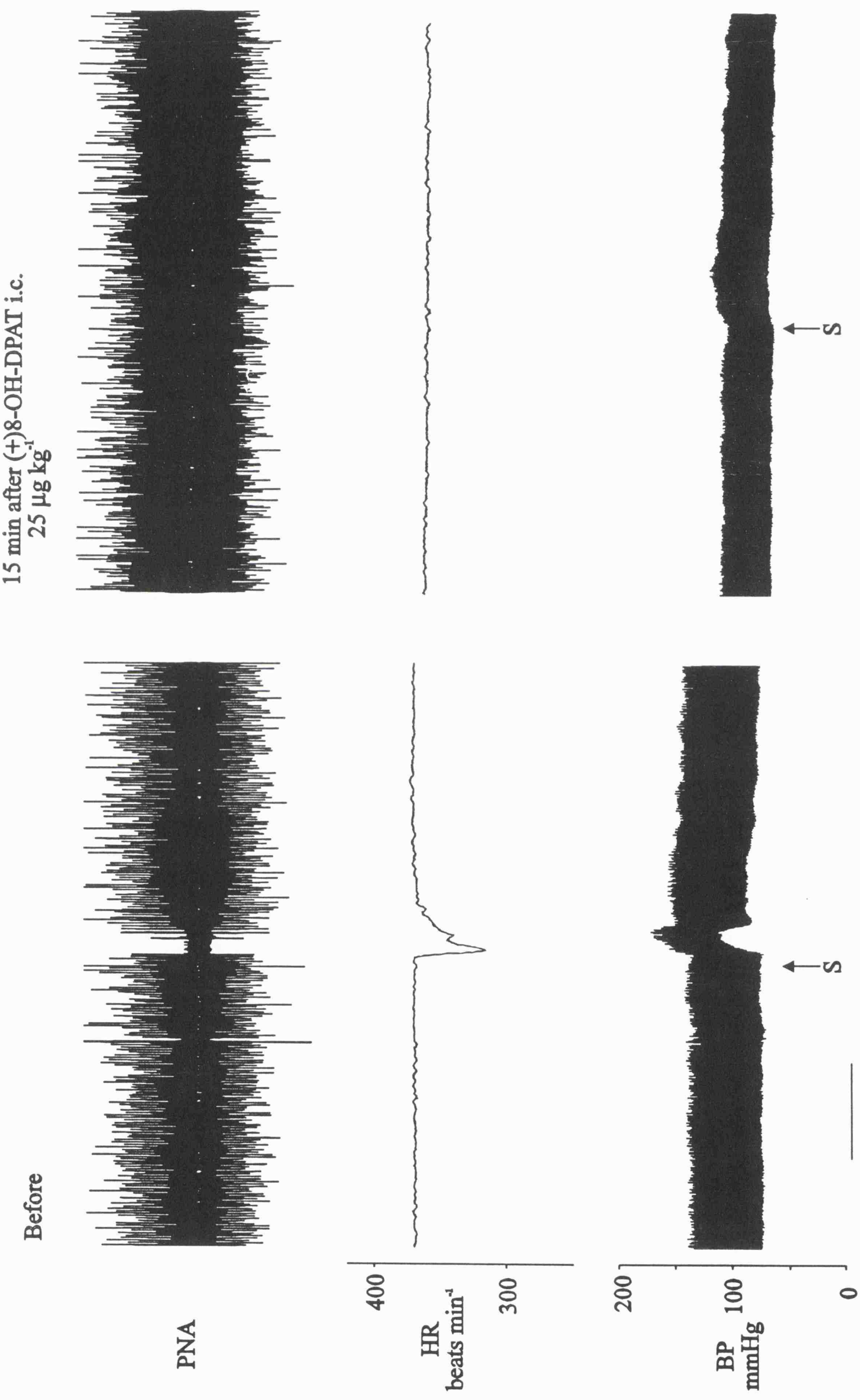

Before
15 min after buspirone i.c.
200 μg kg-1
PNA
HR
beats min-1
400
300
BP
mmHg
200
100
0
S
S
20s

Figure 3.27

Trace shows the response to smoke delivered to the upper airways, in an atenolol (1 mg kg^{-1}; i.v.) pretreated rat, 5 min before and 15 min after administration of 25 $\mu g\ kg^{-1}$ (+)8-OH-DPAT i.c.

From the top, the traces illustrate phrenic nerve activity (PNA), heart rate (HR) and blood pressure (BP). Each trace is 120 s in duration.

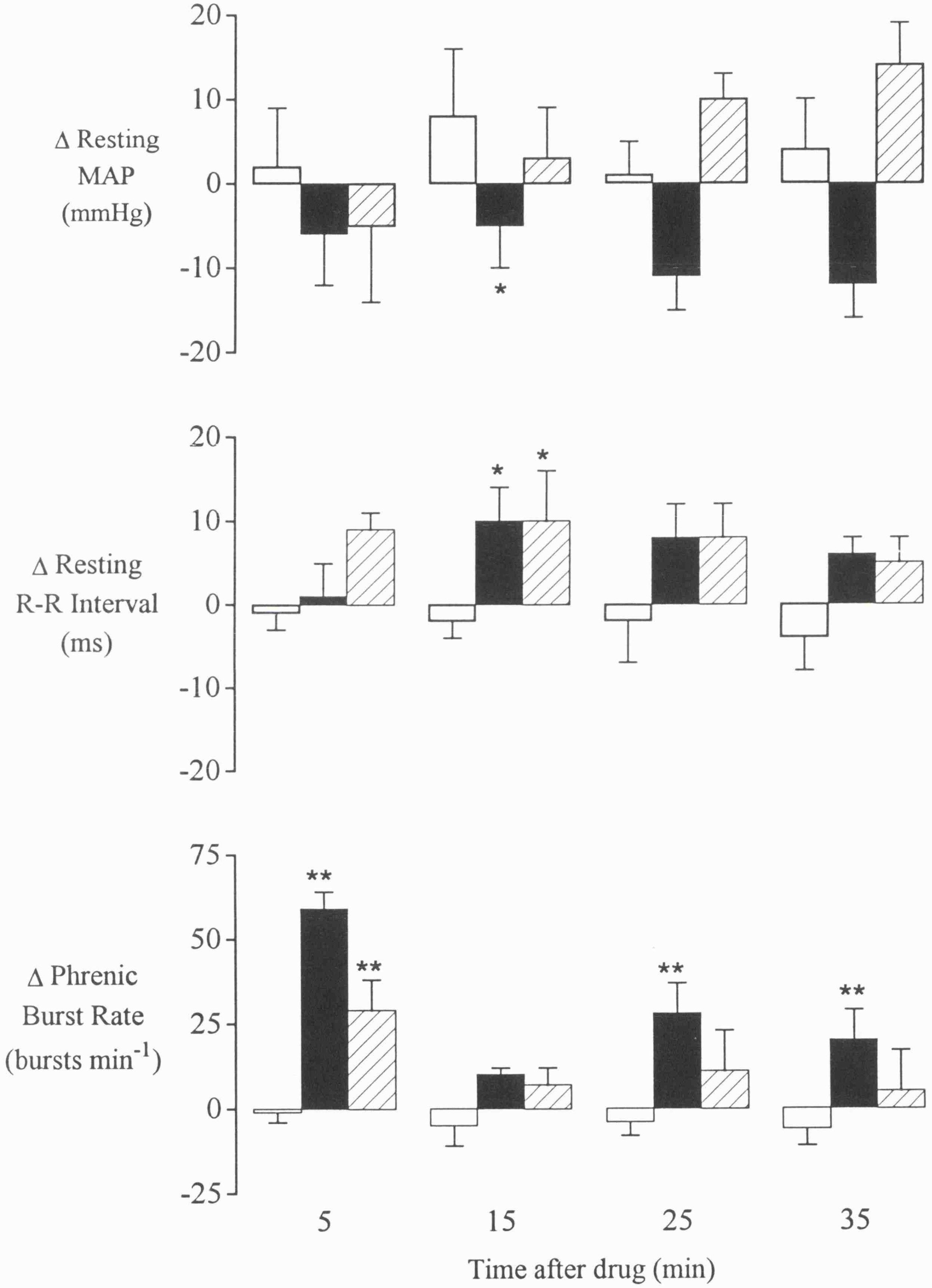
A bolus of smoke was passed through the upper airways at the point marked by an arrow and the letter S.



Before
15 min after (+)8-OH-DPAT i.c.
25 µg kg-1
PNA
HR
beats min-1
400
300
BP
mmHg
200
100
0
S
S
20s

Figure 3.28 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rats: histograms showing changes (Δ) in resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms) and phrenic nerve burst rate (bursts min^{-1}) 5 min after intracisternal (i.c.) injections of saline (20 μl; □ ; n=5), (+)8-OH-DPAT (25 μg kg^{-1}; ■ ; n=5) and buspirone (200 μg kg^{-1}; ▨ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by drug have been compared with those caused by saline using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.



Δ Resting MAP (mmHg)
20
10
0
-10
-20
*
Δ Resting R-R Interval (ms)
20
10
0
-10
-20
* *
Δ Phrenic Burst Rate (bursts min⁻¹)
75
50
25
0
-25
** ** ** **
5
15
25
35
Time after drug (min)

Figure 3.29 Anaesthetised, atenolol (i.v. 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rats: histograms showing changes (Δ) in the reflex increases in mean arterial blood pressure (MAP; mmHg), R-R interval (ms) and apnoea duration (s) elicited by passing smoke through the nasal cavity 5 min after intracisternal (i.c.) injections of saline (20 μl; □ ; n=5), (+)8-OH-DPAT (25 μg kg^{-1}; ■ ; n=5) and buspirone (200 μg kg^{-1}; ▨ ; n=5) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by drug have been compared with those caused by saline using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

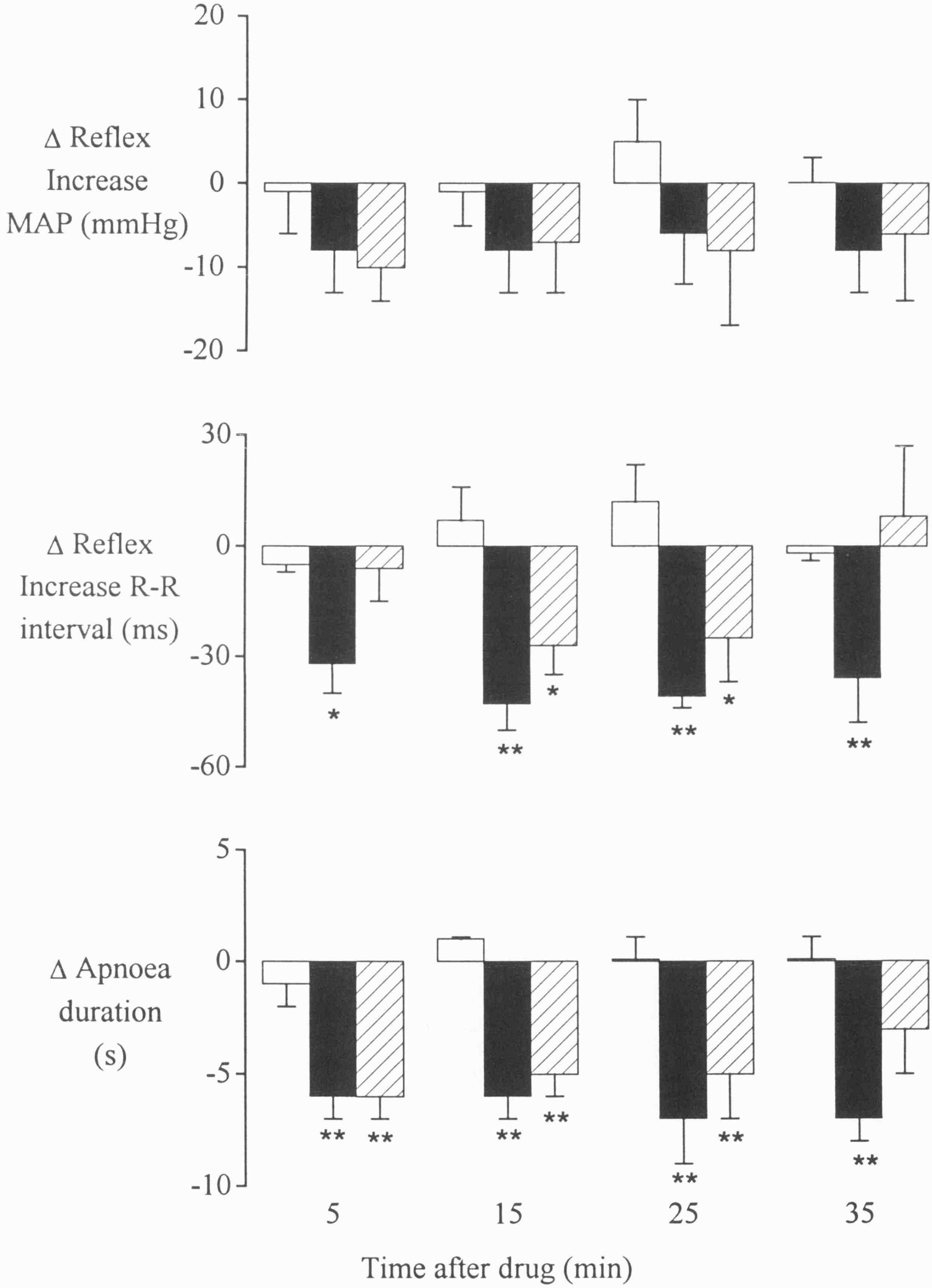
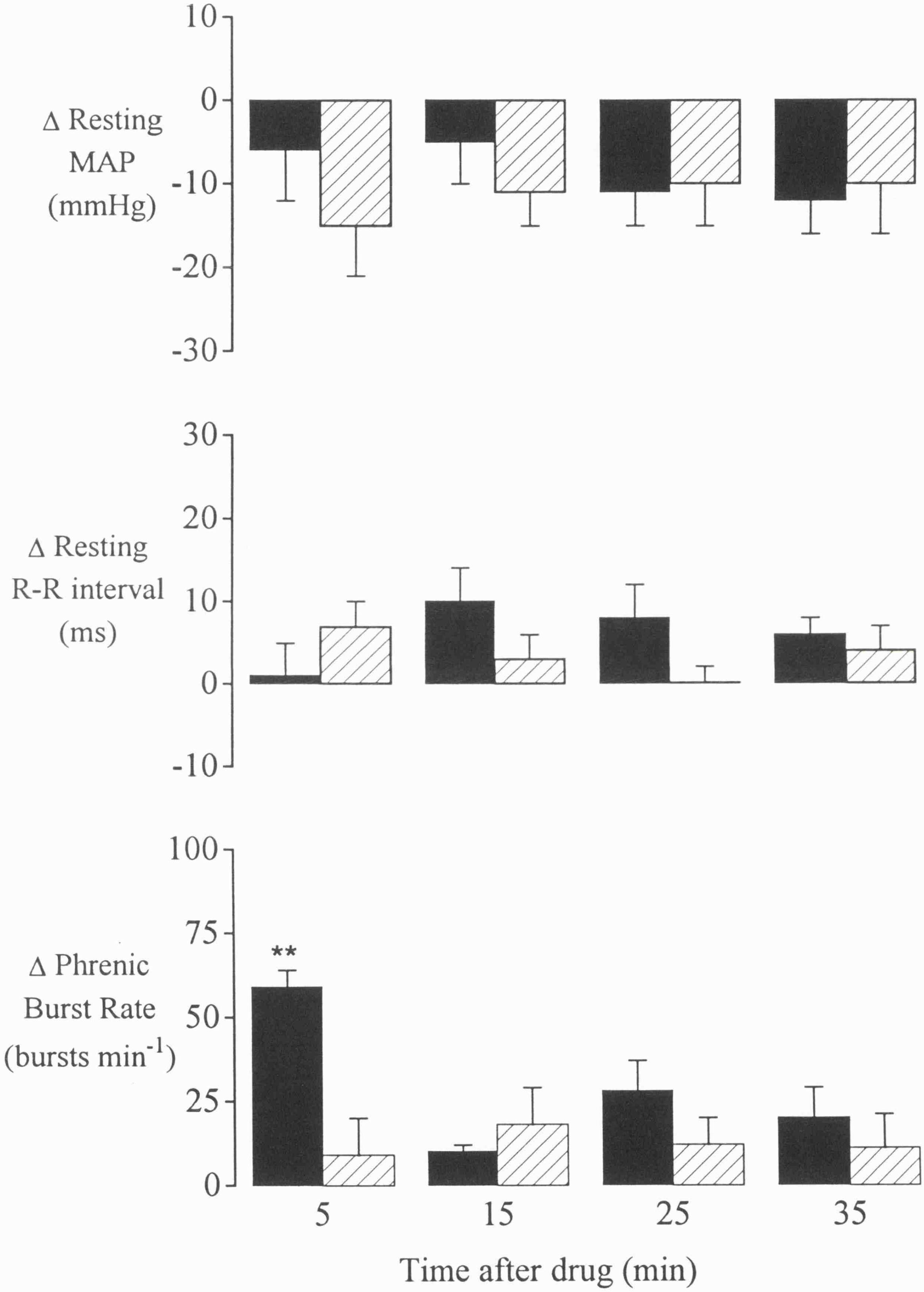

20
10
Δ Reflex
Increase
MAP (mmHg)
0
-10
-20
30
Δ Reflex
Increase R-R
interval (ms)
0
-30
-60
*
**
*
**
*
**
5
Δ Apnoea
duration
(s)
0
-5
-10
** **
** **
** **
**
5
15
25
35
Time after drug (min)

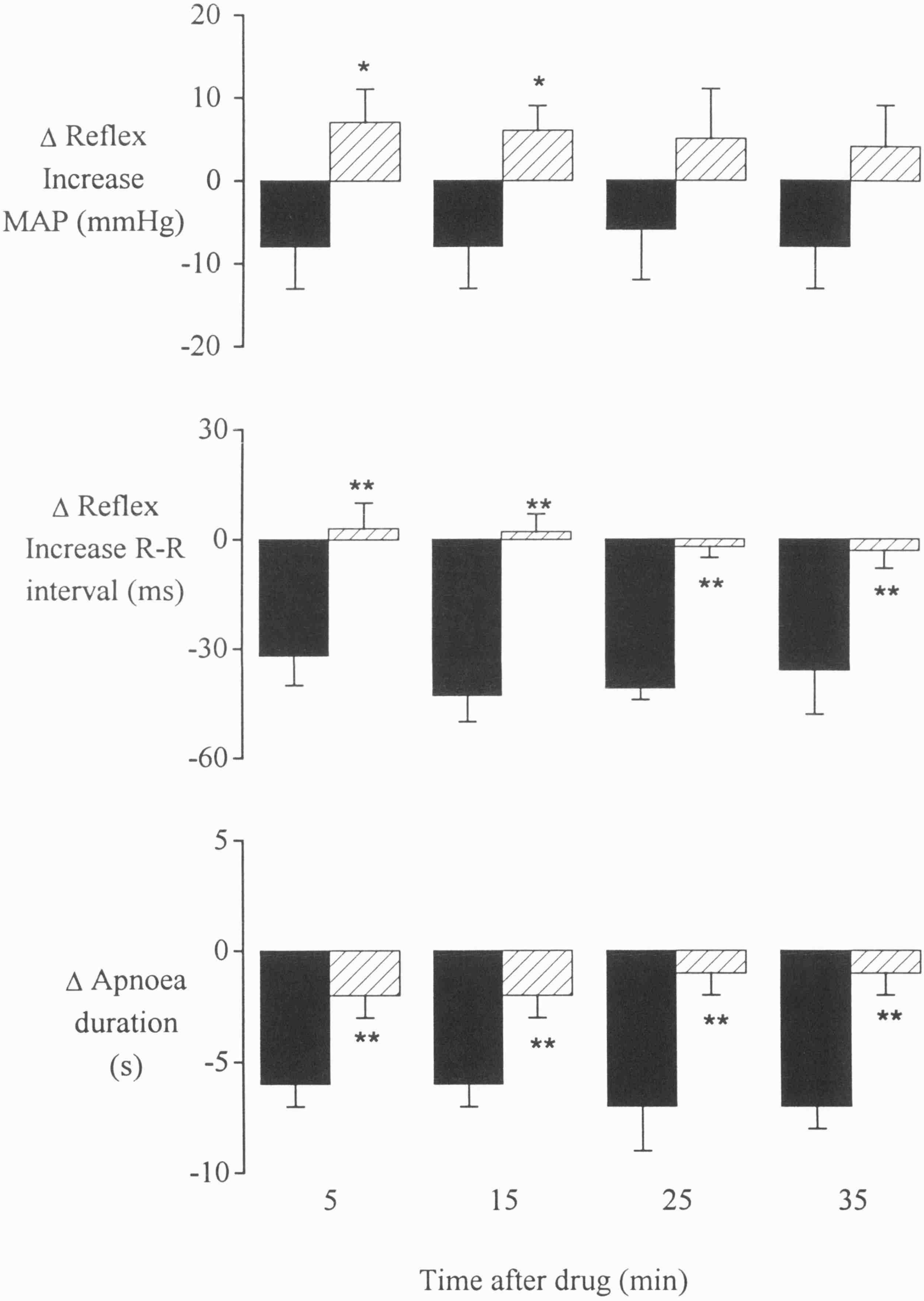
Figure 3.30 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rats: histograms showing changes (Δ) in resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms) and phrenic nerve burst rate (bursts min^{-1}) 5 min after intracisternal (i.c.) injections of (+)8-OH-DPAT (25 μg kg^{-1}; ■ ; n=5) or (+)8-OH-DPAT (25 μg kg^{-1}; ▨ ; n=5) 20 min after pretreatment with WAY-100802 (50 μg kg^{-1}) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by (+)8-OH-DPAT alone have been compared with those caused by (+)8-OH-DPAT after WAY-100802 pretreatment using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.



10
0
-10
-20
-30
Δ Resting
MAP
(mmHg)
30
20
10
0
-10
Δ Resting
R-R interval
(ms)
100
75
50
25
0
**
Δ Phrenic
Burst Rate
(bursts min-1)
5
15
25
35
Time after drug (min)

Figure 3.31 Anaesthetised, atenolol (i.v. 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rats: histograms showing changes (Δ) in the reflex increases in mean arterial blood pressure (MAP; mmHg), R-R interval (ms) and apnoea duration (s) elicited by passing smoke through the nasal cavity 5 min after (+)8-OH-DPAT (i.c.; 25 μg kg^{-1}; ■ ; n=5) or (+)8-OH-DPAT (i.c.; 25 μg kg^{-1}; ▨ ; n=5) 20 min after pretreatment with WAY-100802 (i.c.; 50μg kg^{-1}) and thereafter at 10 min intervals over 35 min. Each column represents the mean change and the bars show s.e. mean. Changes caused by drug have been compared with those caused by saline using ANOVA and least significant difference test. * $p<0.05$; ** $p<0.01$.



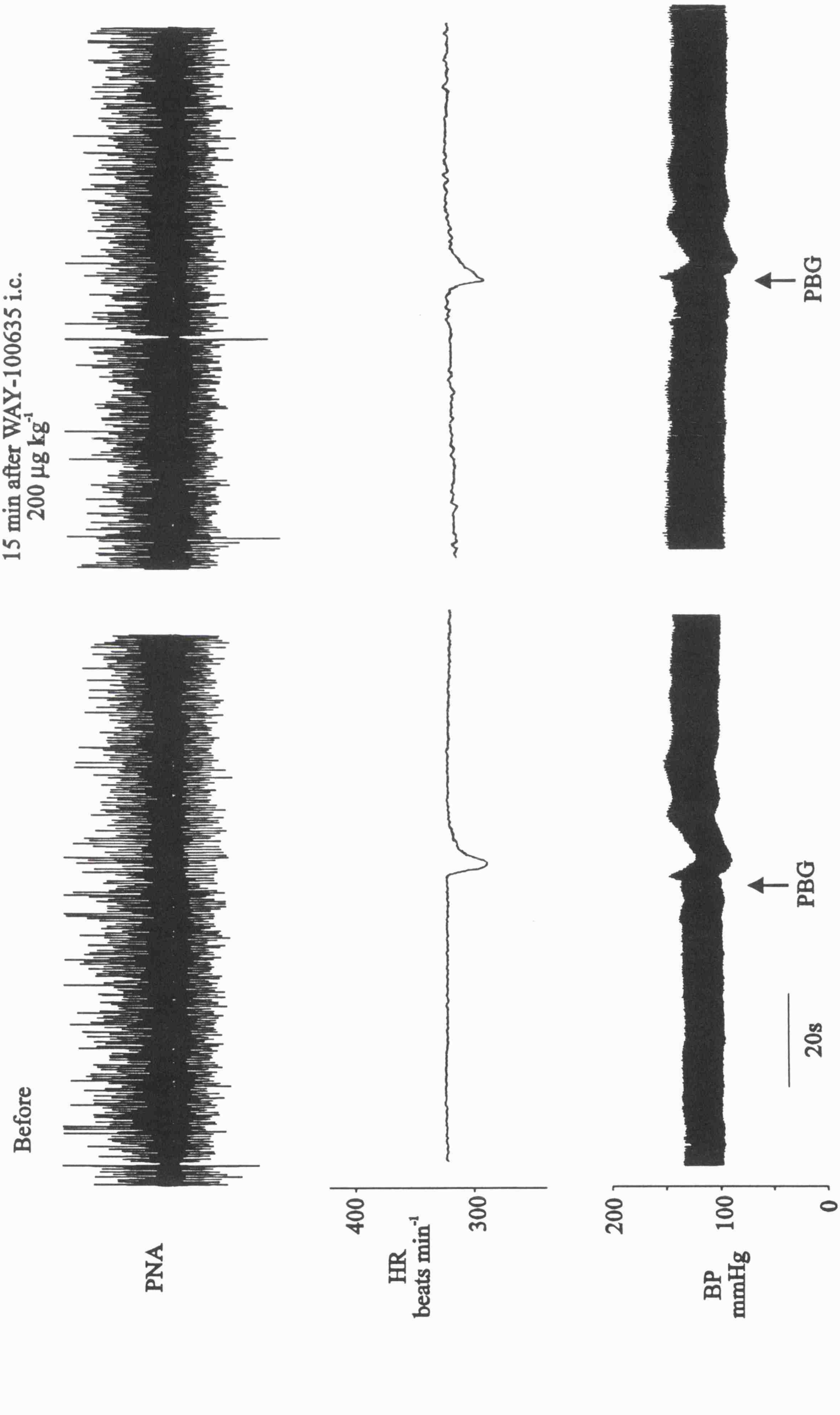
20
10
0
-10
-20
*
*
Δ Reflex
Increase
MAP (mmHg)
30
0
-30
-60
**
**
**
**
Δ Reflex
Increase R-R
interval (ms)
5
0
-5
-10
**
**
**
**
Δ Apnoea
duration
(s)
5
15
25
35
Time after drug (min)

Figure 3.32

Trace shows the response to phenylbiguanide (10 - 30 μg kg^{-1}) injected into the right atrium, in atenolol (1 mg kg^{-1}; i.v.) pretreated rats, 5 min before and 15 after administration of 200 μg kg^{-1} WAY-100635 i.c.

From the top, the traces illustrate phrenic nerve activity (PNA), heart rate (HR) and blood pressure (BP). Each trace is 120 s in duration.

A bolus of phenylbiguanide was injected into the right atrium at the point marked by an arrow and the letters PBG.



Before
15 min after WAY-100635 i.c.
200 µg kg-1
PNA
HR
beats min-1
400
300
BP
mmHg
200
100
0
PBG
PBG
20s

Table 3.19a Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rats: showing the absolute values (mean ± s.e. mean) of resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min before and 5 min after administration of saline (25 μl; i.c.; n=5) or buspirone (200 μg kg^{-1}; i.c.; n=5) and thereafter at 10 min intervals over 35 min. The changes (Δ; mean ± s.e. mean) in resting values of the variables are given in parentheses, calculated from the values at 5 min before administration of the test substances. Time matched values of the drug versus vehicle have been compared using ANOVA and least significant difference test.

* p<0.C5; ** p<0.01.

Table 3.19a sal-busp base

Saline 20 μl i.c. n=5.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	75 ± 9	78 ± 9 **(2±7)**	83 ± 6 **(8±8)**	77 ± 7 **(1±4)**	80 ± 5 **(4±6)**
R-R interval (ms)	167 ± 6	166 ± 5 **(-1±2)**	165 ± 5 **(-2±2)**	166 ± 7 **(-2±5)**	164 ± 5 **(-4±4)**
Phrenic burst rate(bursts min^{-1})	116 ± 8	115 ± 7 **(-1±3)**	111 ± 3 **(-5±6)**	112 ± 6 **(-4±4)**	110 ± 6 **(-6±5)**

Buspirone 200 μg kg^{-1} i.c. n=5.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	74 ± 7	69 ± 8 **(-5±9)**	77 ± 4 **(3±6)**	84 ± 8 **(10±3)**	88 ± 5 **(14±5)**
R-R interval (ms)	167 ± 5	177 ± 6 **(9±2)**	178 ± 7 **(10±6*)**	176 ± 6 **(8±4)**	172 ± 6 **(5±3)**
Phrenic burst rate(bursts min^{-1})	123 ± 5	152 ± 11**(29±9**)**	130 ± 5 **(7±5)**	134 ± 13 **(11±12)**	128 ± 14 **(5±12)**

Table 3.19b Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rats: showing the absolute values (mean ± s.e. mean) of resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min before and 5 min after administration of saline (25 µl; i.c.; n=5) or (+)8-OH-DPAT (25 µg kg^{-1}; i.c.; n=5) and thereafter at 10 min intervals over 35 min. The changes (Δ; mean ± s.e. mean) in resting values of the variables are given in parentheses, calculated from the values at 5 min before administration of the test substances. Time matched values of the drug versus vehicle have been compared using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.19b Sal-DPAT base

Saline 20 μl i.c. n=5.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	75 ± 9	78 ± 9 **(2±7)**	83 ± 6 **(8±8)**	77 ± 7 **(1±4)**	80 ± 5 **(4±6)**
R-R interval (ms)	167 ± 6	166 ± 5 **(-1±2)**	165 ± 5 **(-2±2)**	166 ± 7 **(-2±5)**	164 ± 5 **(-4±4)**
Phrenic burst rate(bursts min^{-1})	116 ± 8	115 ± 7 **(-1±3)**	111 ± 3 **(-5±6)**	112 ± 6 **(-4±4)**	110 ± 6 **(-6±5)**

(+)8-OH-DPAT 25 μg kg^{-1} i.c. n=5.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	86 ± 4	80 ± 5 **(-6±6)**	80 ± 5 **(-5±5)**	75 ± 3 **(-11±4)**	73 ± 1 **(-12±4*)**
R-R interval (ms)	167 ± 1	168 ± 4 **(1±4)**	177 ± 4 **(10±4*)**	175 ± 4 **(8±4)**	173 ± 1 **(6±2)**
Phrenic burst rate(bursts min^{-1})	124 ± 4	177 ± 2** **(59±5**)**	134 ± 4* **(10±0)**	152 ± 9** **(28±9**)**	144 ± 10** **(20±9**)**

Table 3.20a Anaesthetised, atenolol (i.v.; 1 mg kg) pretreated, normoxic, spontaneously breathing rats: showing the absolute reflex changes (mean ± s.e. mean) in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min before and 5 min after administration of saline (20 μl; i.c.; n=5) or buspirone (200 μg kg^{-1} i.c.; n=5) and thereafter at 10 min intervals over 35 min. Changes (Δ; mean ± s.e. mean) in the reflex response of the variables from preinjection (-5 min) values are given. Time matched comparison of drug versus vehicle have been made using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.20a

Saline 20 µl i.c. n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	47 ± 6	46 ± 10 **(-1±5)**	46 ± 7 **(-1±4)**	52 ± 7 **(5±5)**	48 ± 7 **(0±3)**
Increase R-R interval (ms)	25 ± 4	20 ± 3 **(-5±2)**	32 ± 6 **(7±9)**	37 ± 12 **(12±10)**	23 ± 4 **(-2±2)**
Apnoea duration (s)	6 ± 1	5 ± 0 **(-1±1)**	6 ± 1 **(1±0)**	6 ± 2 **(0±1)**	6 ± 1 **(0±1)**

Buspirone 200 µg kg^{-1} i.c. n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	38 ± 3	28 ± 5 **(-10±4)**	31 ± 4 **(-7±6)**	30 ± 8* **(-8±9)**	32 ± 8 **(-6±8)**
Increase R-R interval (ms)	38 ± 15	33 ± 23 **(-6±9)**	12 ± 9 **(-27±8*)**	14 ± 8 **(-25±12*)**	48 ± 33 **(8±19)**
Apnoea duration (s)	7 ± 1	1 ± 1** **(-6±1**)**	2 ± 1** **(-5±1**)**	2 ± 1** **(-5±2**)**	4 ± 1 **(-3±2)**

Table 3.20b Anaesthetised, atenolol (i.v.; 1 mg kg) pretreated, normoxic, spontaneously breathing rats: showing the absolute reflex changes (mean ± s.e. mean) in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min before and 5 min after administration of saline (20 µl; i.c.; n=5) or (+)8-OH-DPAT (25 µg kg^{-1}; i.c.; n=5) and thereafter at 10 min intervals over 35 min. Changes (Δ; mean ± s.e. mean) in the reflex response of the variables from preinjection (-5 min) values are given. Time matched comparison of drug versus vehicle have been made using ANOVA and least significant difference test. * $p<0.05$; ** $p<0.01$.

Table 3.20b

Saline 20 μl i.c. n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	47 ± 6	46 ± 10 **(-1±5)**	46 ± 7 **(-1±4)**	52 ± 7 **(5±5)**	48 ± 7 **(0±3)**
Increase R-R interval (ms)	25 ± 4	20 ± 3 **(-5±2)**	32 ± 6 **(7±9)**	37 ± 12 **(12±10)**	23 ± 4 **(-2±2)**
Apnoea duration (s)	6 ± 1	5 ± 0 **(-1±1)**	6 ± 1 **(1±0)**	6 ± 2 **(0±1)**	6 ± 1 **(0±1)**

(+)8-OH-DPAT 25 μg kg^{-1} i.c. n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	34 ± 4	27 ± 4* **(-8±5)**	26 ± 5* **(-8±5)**	28 ± 6* **(-6±6)**	26 ± 6* **(-8±5)**
Increase R-R interval (ms)	45 ± 6	13 ± 9 **(-32±8*)**	1 ± 5** **(-43±7**)**	4 ± 6** **(-41±3**)**	9 ± 13 **(-36±12**)**
Apnoea duration (s)	7 ± 1	0 ± 0** **(-6±1**)**	1 ± 1** **(-6±1**)**	0 ± 0** **(-7±2**)**	0 ± 0** **(-7±1**)**

Table 3.21 Anaesthetised, atenolol (i.v.; 1 mg kg^{-1}) pretreated, normoxic, spontaneously breathing rats: showing the absolute values (mean ± s.e. mean) of resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms), phrenic burst rate (bursts min^{-1}) and renal nerve activity (RNA; %) 5 min before and 5 min after administration of (+)8-OH-DPAT (25 μg kg^{-1}; i.c.; n=5) or (+)8-OH-DPAT (25 μg kg^{-1}; i.c.; n=5) 20 min after pretreatment with WAY-100802 (50 μg kg^{-1}; i.c.)and thereafter at 10 min intervals over 35 min. The changes (Δ; mean ± s.e. mean) in resting values of the variables are given in parentheses, calculated from the values at 5 min before administration of the test substances. Time matched values of the (+)8-OH-DPAT versus (+)8-OH-DPAT after WAY-100802 pretreatment have been compared using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.21

(+)8-OH-DPAT 25 μg kg^{-1} i.c. n=5.

Time after (+)8-OH-DPAT (min)	-5	5	15	25	35
MAP (mmHg)	86 ± 4	80 ± 5 **(-6±6)**	80 ± 5 **(-5±5)**	75 ± 3 **(-11±4)**	73 ± 1 **(-12±4)**
R-R interval (ms)	167 ± 1	168 ± 4 **(1±4)**	177 ± 4 **(10±4)**	175 ± 4 **(8±4)**	173 ± 1 **(6±2)**
Phrenic burst rate(bursts min^{-1})	124 ± 4	177 ± 2 **(59±5)**	134 ± 4 **(10±0)**	152 ± 9 **(28±9)**	144 ± 10 **(20±9)**

(+)8-OH-DPAT 25 μg kg^{-1} i.c. (WAY-100802 50 μg kg^{-1} i.c. pretreated 20 minutes previously) n=5.

Time after (+)8-OH-DPAT (min)	-5	5	15	25	35
MAP (mmHg)	94 ± 3	78 ± 7 **(-15±6)**	82 ± 6 **(-11±4)**	83 ± 5 **(-10±5)**	84 ± 5 **(-10±6)**
R-R interval (ms)	171 ± 3	178 ± 5 **(7±3)**	174 ± 5 **(3±3)**	171 ± 5 **(0±2)**	175 ± 5 **(4±3)**
Phrenic burst rate(bursts min^{-1})	114 ± 7	123 ± 13** **(9±11**)**	134 ± 13 **(18±11)**	126 ± 11 **(12±8)**	125 ± 13 **(11±10)**

Table 3.22 Anaesthetised, atenolol (i.v.; 1 mg kg) pretreated, normoxic, spontaneously breathing rats: showing the absolute reflex changes (mean ± s.e. mean) in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA; %) elicited by passing smoke through the nasal cavity 5 min before and 5 min after administration of (+)8-OH-DPAT (25 $\mu g\ kg^{-1}$; i.c.; n=5) or (+)8-OH-DPAT (25 $\mu g\ kg^{-1}$; i.c.; n=5) 20 minutes after pretreatment with WAY-100802 (50 $\mu g\ kg^{-1}$; i.c.) and thereafter at 10 min intervals over 35 min. Changes (Δ; mean ± s.e. mean) in the reflex response of the variables from preinjection (-5 min) values are given. Time matched comparison of drug versus vehicle have been made using ANOVA and least significant difference test.

* $p<0.05$; ** $p<0.01$.

Table 3.22

(+)8-OH-DPAT 25 μg kg-1 i.c. n=5.

Time after (+)8-OH-DPAT (min)	-5	5	15	25	35
Increase MAP (mmHg)	34 ± 4	27 ± 4 **(-8±5)**	26 ± 5 **(-8±5)**	28 ± 6 **(-6±6)**	26 ± 6 **(-8±5)**
Increase R-R interval (ms)	45 ± 6	13 ± 9 **(-32±8)**	1 ± 5 **(-43±7)**	4 ± 6 **(-41±3)**	9 ± 13 **(-36±12)**
Apnoea duration (s)	7 ± 1	0 ± 0 **(-6±1)**	1 ± 1 **(-6±1)**	0 ± 0 **(-7±2)**	0 ± 0 **(-7±1)**

(+)8-OH-DPAT 25 μg kg-1 i.c. (WAY-100802 50 μg kg-1 i.c. pretreated 20 minutes previously), n=5.

Time after (+)8-OH-DPAT (min)	-5	5	15	25	35
Increase MAP (mmHg)	44 ± 6	50 ± 8** **(7±4*)**	49 ± 6** **(6±3*)**	48 ± 5* **(5±6)**	47 ± 5 **(4±5)**
Increase R-R interval (ms)	30 ± 4	32 ± 8 **(3±7**)**	31 ± 6** **(2±5**)**	28 ± 1* **(-2±3**)**	26 ± 3 **(-3±5**)**
Apnoea duration (s)	7 ± 1	6 ± 1** **(-2±1**)**	6 ± 1** **(-2±1**)**	6 ± 1** **(-1±1**)**	6 ± 1** **(-1±1**)**

Table 3.23 Summary of results obtained in spontaneously breathing, normoxic rats.

Resting. Effects of drugs on baseline R-R interval (R-R), phrenic nerve rate (PNA rate) and mean arterial blood pressure (MAP).

Reflex. Effects of drugs on reflex increase in R-R interval (R-R), apnoea duration (apnoea) and reflex increase in mean arterial blood pressure (MAP).

0 = No change.

(↑) = Significant increase more than 15 min after drug administration.

↑ = Significant increase at only one time interval, within 15 min of drug administration.

↑↑ = Significant increase at more than one time interval, within 15 min of drug administration.

(↓) = Significant decrease more than 15 min after drug administration.

↓ = Significant decrease at only one time interval, within 15 min of drug administration.

↓↓ = Significant decrease at more than one time interval, within 15 min of drug administration.

Table 3.23 Summary of results; spontaneously breathing, normoxic rats.

Resting.

Drug	R-R	PNA rate	MAP
Buspirone i.c.	↑	↑	0
(+)8-OH-DPAT i.c.	↑	↑↑	(↓)
(+)8-OH-DPAT i.c./WAY100802 i.c.	0	↑	0
WAY-100635 i.c.	0	↓	↓
WAY-100802 i.c.	0	↓	0
(-)Pindolol i.c.	0	↓↓	0
Granisetron i.c.	0	0	0

Reflex.

Drug	R-R	Apnoea	MAP
Buspirone i.c.	↓	↓↓	0
(+)8-OH-DPAT i.c.	↓↓	↓↓	0
(+)8-OH-DPAT i.c./WAY100802 i.c.	0	0	↑↑
WAY-100635 i.c.	0	↑	0
WAY-100802 i.c.	0	↑	0
(-)Pindolol i.c.	↓	0	↓↓
Granisetron i.c.	0	0	0

Summary of results.

Effects of 5-HT_{1A} Receptor Agonists.

Administration (i.c.) of buspirone or (+)8-OH-DPAT caused a significant increase in resting R-R interval and phrenic burst rate and (+)8-OH-DPAT also reduced baseline MAP. Both drugs inhibited the reflex increase in R-R interval and apnoea duration.

Effects of 5-HT_{1A} Receptor Antagonists.

Administration (i.c.) of WAY-100635, WAY-100802 and (-)pindolol caused a reduction of phrenic burst rate and WAY-100635 also reduced resting MAP. Both WAY-100635 and WAY-100802 inhibited the reflex apnoea duration without altering the other reflex responses. In contrast, (-)pindolol inhibited the reflex increases in R-R interval and MAP but did not alter the apnoea duration. Pretreatment with WAY-100802 (i.c.) before administration of (+)8-OH-DPAT (i.c.) inhibited both the baseline and reflex effects of this drug but there was then a significant potentiation of the reflex increase in MAP.

Effects of a 5-HT_3 Receptor Antagonist, granisetron.

Administration (i.c.) of granisetron had no effect on either the baseline or reflex variables.

Discussion

Evidence that the dose of atenolol used was adequate to block the effects of cardiac sympathetic activity in the rabbit and rat.

All the present experiments were carried out in atenolol (i.v.) pretreated urethane anaesthetised rabbits and rats. The purpose of atenolol (1 mg kg^{-1}) pretreatment was to block the sympathetic drive to the heart, so changes in heart rate i.e. R-R interval would be indicative of changes in cardiac vagal motoneurone activity. The β_1-adrenoceptor antagonist, atenolol was chosen because it does not bind to 5-HT receptors (Middlemis et al., 1977) and it poorly penetrates the central nervous system (Street et al., 1979). To check that the dose of atenolol was adequate to block sympathetic drive to the heart over the period of the experiment (80 - 120 min) atropine methylnitrate was given at the end of some experiments and the smoke stimulus was repeated. It was found that this smoke stimulus did not evoke a reflex increase in R-R interval. In addition, in some experiments the carotid artery was bilaterally occluded for 45 s, before, approximately 10 min after administration of atenolol and 5 min after atropine. Again, the tachycardia was greatly attenuated by atenolol, and abolished after atropine. These data indicate that this dose of atenolol was sufficient to block the sympathetic β_1-adrenoceptor mediated drive to the heart over the duration of the experiments.

Evidence that 5-HT_{1A} receptors play a role in the reflex activation of cardiac vagal motor neurones in rabbits.

Effects of 5-HT_{1A} receptor agonists on resting variables.

Administration of the 5-HT_{1A} receptor agonists (+)8-OH-DPAT, buspirone and nn-DP-5-CT (see Hoyer & Fozard, 1991) i.c. to atenolol pretreated, anaesthetised rabbits caused significant increases in R-R interval, rate of phrenic nerve bursts and a decrease in renal nerve activity. Previous studies have found that administration of 8-OH-DPAT and other 5-HT_{1A} agonists in rabbits (Hof & Fozard, 1989; Shepheard et al., 1990a), rats (Gradin et al., 1985; Fozard et al., 1987) and cats (Ramage & Fozard, 1987; McCall et al., 1987; Ramage et al., 1988) also caused a bradycardia however this was associated with hypotension. The failure to see a decrease in blood pressure is probably due to the relatively low resting mean arterial blood pressure in the anaesthetised rabbits in the present experiments (52 ± 7 mmHg) compared to 71 ± 4 mmHg (Hof & Fozard, 1989) and 80 ± 3 mmHg (Shepheard et al., 1990), the latter in urethane anaesthetised rabbits. An explanation for the lower blood pressures is that in the present experiments the animals are pretreated with atenolol, which caused a significant fall in resting mean arterial blood pressure (see Appendix 5.21) and therefore may have masked any hypotensive effect of the 5-HT_{1A} receptor agonists used in the present study. However the present results are consistent with the ability of 5-HT_{1A} receptor agonists to increase respiratory drive in rabbits (Shepheard et al., 1990), rats (Sporton et al., 1990) and cats (Gillis et al., 1989). Further, the ability of intravenous pretreatment with the highly selective 5-HT_{1A} receptor antagonist WAY-100635 (Forster et al., 1995) to block all of the baseline effects of buspirone i.c. and (+)8-OH-DPAT i.c. confirms that the above changes are due to activation of 5-HT_{1A} receptors and by inference also those of nn-DP-5-CT.

Effects of 5-HT_{1A} receptor antagonists on resting variables.

In the present study the 5-HT_{1A} receptor antagonists WAY-100635 and (-)pindolol were administered (i.c.). WAY-100635 caused no significant changes to any of the baseline variables, while (-)pindolol caused a significant increase in resting R-R interval and reduced phrenic burst rate and renal nerve activity. It has been demonstrated that WAY-100635 has a much greater affinity for 5-HT_{1A} receptors over other receptor types and has no partial agonist effects at either 5-HT_{1A} somatodendritic autoreceptors or 5-HT_{1A} heteroreceptors (Forster et al., 1995). In contrast (-)pindolol is a partial agonist at 5-HT_{1A} receptors (Hjorth & Carlsson, 1986) as well as having partial agonist actions at β-adrenoceptors (Hicks et al., 1987) and in addition has actions at 5-HT_{1B} receptors, although it has only low affinity for 5-HT_{1D} receptors(see Hoyer & Fozard, 1991). It is possible that the absence of baseline effects by WAY-100635 were due to insufficient blockade of central 5-HT_{1A} receptors. However, this is very unlikely as even when this dose (100 μg kg^{-1}) of WAY-100635 was given i.v. it was sufficient to block the effects of buspirone (i.c.) or (+)8-OH-DPAT (i.c.). Therefore, these differences between

(-)pindolol and WAY-100635 are probably due to the lack of selectivity of (-)pindolol. In addition as WAY-100635 failed to alter the baseline variables it can be concluded that there was very little basal tone at brain stem 5-HT_{1A} receptors controlling cardiac vagal motoneurones, respiratory and renal sympathetic outflow in the absence of reflex activity.

Effects of 5-HT_{1A} receptor antagonists on the "diving response".

Administration (i.c.) of WAY-100635 or (-)pindolol caused a substantial inhibition of the reflex bradycardia and significantly reduced the reflex hypertension. In addition (-)pindolol also significantly reduced the apnoea duration and reflex renal sympathoexcitation. These data therefore indicate that the effects of reflex bradycardia and hypertension of the "diving response" evoked by smoke stimulation of the upper airways are mediated by activation of central 5-HT_{1A} receptors. The failure of the same dose of WAY-100635 administered i.v. to affect the "diving response" indicates that this is due to a central action of WAY-100635 and by inference also that of (-)pindolol. These observations are consistent with previous results obtained by Futuro-Neto et al, 1993, in the rabbit and Bogle et al., 1990, in the rat indicating that the 5-HT_{1A} receptors are modulating the reflex activation of preganglionic cardiac vagal motoneurones. However, this data indicates that there is no species difference between the role of 5-HT_{1A} receptors in rabbits and rats as had been suggested by Futuro-Neto et al., 1993 (see later for further discussion).

The observation that (-)pindolol inhibited the apnoea duration may explain why this drug caused a larger inhibition of the reflex bradycardia than WAY-100635, since inspiration both directly inhibits activation of cardiac vagal motoneurones (Gilbey et al., 1984) and inhibits them indirectly by activation of lung stretch receptors (Angell-James & Daly, 1978; see later for further discussion).

The inhibition of the bradycardia caused by these 5-HT_{1A} antagonists would be expected to cause a smaller reduction in cardiac output during the "diving response", thus increasing the reflex hypertension. However, both (-)pindolol and WAY-100635 inhibited the reflex hypertension. In the case of (-)pindolol the additional sympathoinhibitory action (presumably due to the partial agonist action at 5-HT_{1A} receptors of (-)pindolol) would provide a satisfactory explanation for the reduction of this reflex hypertension. However, the observation that WAY-100635 did not inhibit the reflex renal sympathoexcitation suggests that other mechanisms are also involved in the reduction of the reflex hypertension. The weak α_1-adrenoceptor antagonist properties of WAY-100635 could provide one mechanism of action, however, the reduction of the hypertension was certainly not due to the drug leaking out of the brain to have an effect on peripheral α_1-adrenoceptors since i.v. WAY-100635 had no effect. Blockade of central α_1-adrenoceptors is known to cause differential sympathoinhibition, affecting cardiac and splanchnic but not renal outflow (Ramage & Wyllie, 1995). It is of interest that in this respect the effect of WAY-100635 on the reflex increase in blood pressure is weaker than that of (-)pindolol.

Effects of 5-HT_{1A} receptor agonists on the "diving response".

In the present study it was found that (+)8-OH-DPAT (i.c.) inhibited, buspirone (i.c.) potentiated and nn-DP-5-CT (i.c.) had no significant effect on the reflex bradycardia. The effect of (+)8-OH-DPAT was associated with an inhibition of the apnoea duration, whereas neither buspirone nor nn-DP-5-CT changed this variable. In addition (+)8-OH-DPAT and nn-DP-5-CT caused significant inhibitions of the reflex increase in renal nerve activity, while all the drugs inhibited the reflex increase in blood pressure. The buspirone and (+)8-OH-DPAT effects on the reflex bradycardia are in agreement with those obtained by Futuro-Neto et al., 1993. As discussed earlier, all 3 agonists increased baseline R-R interval and this was attributed to activation of central 5-HT_{1A} receptors, therefore it was surprising that only buspirone potentiated the reflex bradycardia. However, the inhibition of the reflex bradycardia caused by (+)8-OH-DPAT could be due to the inhibition of the apnoea produced by this drug (see later discussion).

Despite causing a resting renal sympathoinhibition, buspirone did not significantly change the size of the reflex increase in renal nerve activity. In the light of this observation the reduction in the reflex hypertension can be attributed to the potentiation of the reflex bradycardia causing a more profound reduction of the cardiac output during the reflex. In contrast, the inhibition of the reflex increase in blood pressure produced by (+)8-OH-DPAT and nn-DP-5-CT cannot be attributed to changes in reflex bradycardia, but could be attributed to their ability to inhibit the reflex renal sympathoexcitation.

Do buspirone and (+)8-OH-DPAT cause their effects on the "diving response" through activation of 5-HT$_{1A}$ receptors?

Pretreatment (i.v.) with WAY-100635 significantly attenuated the effects of buspirone on the "diving response", but only significantly attenuated the (+)8-OH-DPAT mediated inhibition of the apnoea and reflex renal sympathoexcitation. Therefore the ability of buspirone to potentiate the reflex bradycardia and (+)8-OH-DPAT to inhibit the apnoea and renal sympathoexcitation is due to activation of 5-HT$_{1A}$ receptors. The absence of inhibition of the reflex renal sympathoinhibition and apnoea suggests that buspirone is unable to activate the 5-HT$_{1A}$ receptors controlling these variables. This may reflect differences in the sensitivity of these 5-HT$_{1A}$ receptors for buspirone, or buspirone may be having other actions which mask the changes caused by activation of the 5-HT$_{1A}$ receptors controlling these variables. Buspirone is known to have partial agonist activity at the 5-HT$_{1A}$ receptor (Schoeffter & Hoyer, 1988). Therefore, the inability of buspirone to change the reflex renal sympathoexcitation could be explained by a partial agonist action at the 5-HT$_{1A}$ receptors involved in the control of renal sympathetic outflow. However, (-)pindolol also has a partial agonist action at 5-HT$_{1A}$ receptors (Schoeffter & Hoyer, 1988) and the reflex renal sympathoexcitation was attenuated by this drug (see above). Therefore, other mechanisms must be involved. The ability of both (+)8-OH-DPAT (Glaser et al., 1991) and nn-DP-5-CT (Schoeffter & Hoyer, 1988) to inhibit the reflex increase in renal nerve activity is consistent with them being full agonists at 5-HT$_{1A}$ receptors. However, the failure of (+)8-OH-DPAT and nn-DP-5-CT to potentiate the reflex bradycardia suggests they are having other actions in addition to activation of 5-HT_{1A} receptors.

It could be argued that the (+)8-OH-DPAT evoked inhibition of the reflex bradycardia is mediated through the effect of the drug on the apnoea duration. It has already been established that stimulation of lung stretch receptors during the "diving response" can inhibit or even reverse the reflex bradycardia and vasoconstriction (Angell-James & Daly, 1978) and also that cardiac vagal motoneurones are refractory to excitatory input during inspiration (Gilbey et al., 1984). Figure 3.1 in the results section illustrates the point that the "diving response" bradycardia is terminated by the resumption of regular inspiratory activity, with the greatest increase in R-R interval occurring just before the first phrenic burst. Therefore, one explanation for the inhibition of the reflex bradycardia could be that the 8-OH-DPAT mediated reduction in the apnoea duration caused inhibition of the cardiac vagal motoneurones earlier in the development of the response.

Evidence that (+)8-OH-DPAT is not inhibiting the reflex bradycardia by shortening the apnoea duration.

Measurement of the change in R-R interval 3 s after smoke was first introduced into the upper airways avoids the effect of a shorter apnoea curtailing the bradycardia earlier in its development. It was found that 8-OH-DPAT caused no significant change in the bradycardia at 3 s. The failure to see a significant inhibition of the bradycardia at 3 s probably reflects the small size of the bradycardia at this time (9 ± 3 ms 5 min before administration of (+)8-OH-DPAT). Interestingly the effect of buspirone on the reflex increase in R-R interval after 3 s was a significant potentiation. However, pretreatment with WAY-100635 blocked the (+)8-OH-DPAT mediated inhibition of the smoke evoked apnoea and the increase in phrenic rate, yet, had no effect on the inhibition of the reflex bradycardia. This indicated that (+)8-OH-DPAT could inhibit the bradycardia without reducing the apnoea duration. Further, the view that the 8-OH-DPAT mediated inhibition of the reflex bradycardia is not due to the apnoea being inhibited is supported by the observation from a single experiment that in a rabbit artificially hyperventilated until the phrenic nerve ceased firing, administration of 8-OH-DPAT still caused inhibition of the smoke evoked bradycardia. The failure of WAY-100635 to attenuate the (+)8-OH-DPAT mediated inhibition of the reflex bradycardia suggests that this effect was not mediated through activation of 5-HT_{1A} receptors. It is possible that this effect could be due to the activation of 5-HT_{1D} receptors, the 5-HT receptor subtype for which (+)8-OH-DPAT has the second highest affinity ($pK_D = 6.9$; Bruinvels et al., 1992).

Evidence that 5-HT_{1D} receptors play a role in the reflex activation of cardiac vagal motoneurones in rabbits.

Effects of the 5-HT_{1D} receptor agonist, sumatriptan in the presence of WAY-100635 on resting variables.

Previous research has demonstrated that administration of the 5-HT_{1D} receptor agonist sumatriptan into the IV^{th} ventricle of anaesthetised cats causes a sympathoinhibition and fall in blood pressure (Shepheard et al., 1994). However, owing to the relatively small difference between the affinity of sumatriptan for 5-HT_{1D} and 5-HT_{1A} receptors (about 30 times higher for 5-HT_{1D} receptors; see Hoyer & Fozard, 1991), the authors were unable to attribute these effects to 5-HT_{1D} receptors. Therefore, in the present study, sumatriptan was administered (i.c.) in the presence of WAY-100635 (i.v.), a 5-HT_{1A} receptor antagonist with negligible affinity for 5-HT_{1D} receptors (Forster et al., 1995). In contrast to the results of Shepheard et al., 1994 in the cat, it was found that sumatriptan (i.c.) in the presence of WAY-100635 (i.v.) caused an increase in phrenic burst rate, a sympathoexcitation and an increase in mean arterial blood pressure. Since the dose of WAY-100635 used was sufficient to inhibit all of the effects of buspirone and (+)8-OH-DPAT on resting R-R interval, phrenic burst rate, renal nerve activity and mean arterial blood pressure, it can be concluded that these effects of sumatriptan were due to activation of 5-HT_{1D} receptors. In this respect these data on phrenic burst rate may indicate that 5-HT_{1D} as well as 5-HT_{1A} receptors are involved in the control of respiratory rate, however, it might also reflect an incomplete blockade of the activation of 5-HT_{1A} receptors by sumatriptan.

Effects of the 5-HT$_{1D}$ receptor antagonist, GR-127935 on resting variables.

Administration of the 5-HT$_{1D}$ receptor antagonist GR-127935 (i.c.) caused no significant changes to resting mean arterial blood pressure, phrenic burst rate or renal nerve activity. However, after 25 min GR-127935 caused a small, but significant bradycardia when compared to the distilled water control group. As the dose of GR-127935 did cause significant changes to the "diving response" hypertension and bradycardia (see later), this would suggest that the dose of the drug used was sufficient to interfere with activation of 5-HT$_{1D}$ receptors. Therefore it can be concluded that there was little basal tone at brain stem 5-HT$_{1D}$ receptors controlling cardiac vagal motoneurones, respiratory and renal sympathetic outflow in the absence of reflex activity, as was concluded for 5-HT$_{1A}$ receptors.

Pretreatment with the 5-HT$_{1D}$ receptor antagonist GR-127935 did not change the effect of (+)8-OH-DPAT on the resting variables.

There were no significant differences between the effects of (+)8-OH-DPAT (i.c.) and (+)8-OH-DPAT (i.c.) in the presence of GR-127935 (i.v.) on the resting variables, supporting the above view that the effects of (+)8-OH-DPAT on resting R-R interval and renal sympathetic nerve activity are mediated by activation of 5-HT$_{1A}$ receptors and also indicating that the dose of GR-127935 used does not affect 5-HT$_{1A}$ receptors ($pK_i = 6.9$; Skingle et al., 1990). The absence of a change in the (+)8-OH-DPAT mediated increase in phrenic rate following pretreatment with GR-127935 suggests that activation of 5-HT$_{1D}$ receptors is not involved in this increase. Further, this suggests that the increase in phrenic burst rate seen following administration of sumatriptan in the presence of WAY-100635 was due to incomplete blockade of 5-HT$_{1A}$ receptors.

Effects of the 5-HT$_{1D}$ receptor agonist, sumatriptan in the presence of WAY-100635 on the "diving response".

Administration of sumatriptan (i.c.) in the presence of WAY-100635 (i.v.) caused a significant inhibition of the "diving response" bradycardia and increase in mean arterial blood pressure, without significantly altering the length of the apnoea. Despite causing a large increase in the resting renal nerve activity, sumatriptan did not change the size of the reflex renal sympathoexcitation. From this it is concluded that activation of 5-HT_{1D} receptors can reduce the activation of cardiac vagal motoneurones during the "diving response" and has the opposite effect to activation of 5-HT_{1A} receptors.

Effects of the 5-HT_{1D} receptor antagonist, GR-127935 on the "diving response".
Administration of the 5-HT_{1D} receptor antagonist, GR-127935 i.c. caused a significant potentiation of the "diving response" bradycardia and hypertension, but did not change the reflex renal sympathoexcitation or apnoea duration. Interestingly, there was a large variation in the response to GR-127935 in that in some experiments the drug elicited large potentiations of the reflex (n=3) and in others there was no change (n=2). All of the experiments were performed on rabbits which had "diving responses" that fulfilled the criteria described in the methods. Furthermore when drugs were administered i.c., the injection site was observed using a microscope to ensure that there was no leakage of drug. However, since GR-127935 did not cause any changes to the resting variables there was no positive control to prove that the drug was acting. It is assumed that the drug was active and reached its site of action in all 5 experiments and it is concluded that the 5-HT_{1D} receptors at which GR-127935 is acting are not essential for the reflex to occur. The absence of a change in the response following GR-127935 in 2 experiments suggests there was not necessarily any activity at these receptors during the "diving response", but that when the receptors were stimulated their activity tended to inhibit the reflex bradycardia and hypertension. GR-127935 may be acting to disinhibit the "diving response". Since administration of GR-127935 i.v. had no effect on the reflex, it is concluded that the drug acted at central 5-HT_{1D} receptors.

The effect of (+)8-OH-DPAT in the presence of GR-127935 on the "diving response" bradycardia.
Pretreatment with the 5-HT_{1D} receptor antagonist, GR-127935 (i.v.) abolished the (+)8-OH-DPAT (i.c.) mediated inhibition of the "diving response" bradycardia and renal sympathoexcitation but not the reduction of the apnoea duration and reflex hypertension.

This indicates that the inhibition of the bradycardia caused by (+)8-OH-DPAT is mediated by activation of 5-HT_{1D} receptors. This would explain why (+)8-OH-DPAT, although considered to be the archetypal 5-HT_{1A} receptor agonist, inhibited the reflex bradycardia, when the combined data suggested that activation of 5-HT_{1A} receptors causes excitation of cardiac vagal motoneurones (see above). Further, this emphasises that the effects of (+)8-OH-DPAT cannot be attributed to activation of 5-HT_{1A} receptors alone. Further, it is interesting that the ability of (+)8-OH-DPAT to activate 5-HT_{1D} receptors overrides the ability of this drug to activate 5-HT_{1A} receptors on cardiac vagal motoneurones. It is also surprising that administration of (+)8-OH-DPAT in the presence of GR-127935 did not unmask the predicted 5-HT_{1A} receptor effect of (+)8-OH-DPAT i.e. causing a potentiation of the reflex bradycardia. A possible explanation for this observation is that the dose of GR-127935 was sufficient to block only a high enough proportion of 5-HT_{1D} receptors so that the combined effects of activation by (+)8-OH-DPAT of 5-HT_{1D} and 5-HT_{1A} receptors modulating cardiac vagal motoneurone activity cancelled each other out. This may also explain the observation that nn-DP-5-CT had no effect on the "diving response" bradycardia as this also has affinity for 5-HT_{1D} as well as 5-HT_{1A} receptors (see Hoyer & Fozard, 1991; N.B. buspirone has negligible affinity for 5-HT_{1D} receptors $pK_D = 4.9$).

Pretreatment with GR-127935 (i.v.) attenuated the (+)8-OH-DPAT mediated inhibition of the smoke response renal sympathoexcitation, this was also observed following pretreatment with WAY-100635. Now, GR-127935 has a relatively high affinity for 5-HT_{1A} receptors ($pK_i = 6.9$ for 5-HT_{1A} receptors; Skingle et al., 1993). Therefore, this may indicate that both GR-127935 and WAY-100635 could have inhibited the (+)8-OH-DPAT mediated inhibition of the reflex renal sympathoexcitation by blocking the activation of 5-HT_{1A} receptors. In this respect, WAY-100635, which has a particularly high affinity for 5-HT_{1A} receptors (Forster et al., 1995), had a greater effect than that of GR-127935 (see Table 3.9). Furthermore, the observation that the 5-HT_{1D} receptor agonist, sumatriptan in the presence of WAY-100635 did not inhibit the smoke evoked renal sympathoexcitation, provides further evidence that this effect is not mediated through activation of 5-HT_{1D} receptors. In conclusion the inhibition of the reflex renal sympathoexcitation caused by (+)8-OH-DPAT was mediated by activation of 5-HT_{1A} receptors.

Possible mechanisms by which 5-HT_{1D} receptors modulate cardiac vagal reflexes.

It is known that 5-HT_{1D} receptors are located on serotonergic neurones, where they act as terminal autoreceptors mediating negative feedback control of 5-HT release (Schlicker et al., 1989). This subtype also functions as a heteroreceptor, inhibiting the release of glutamate (Raiteri et al., 1986) and acetylcholine (Harel-Dupas et al., 1991). This suggests 2 possible hypotheses for the mechanism by which 5-HT_{1D} agonists mediate the inhibition of the "diving response" bradycardia. First, stimulation of 5-HT_{1D} receptors may cause presynaptic autoinhibition of 5-HT release and thus indirectly reduce the level of activation of post-synaptic 5-HT_{1A} receptors. Alternatively, the agonists may be activating 5-HT_{1D} heteroreceptors and inhibiting transmission at cholinergic or glutaminergic synapses, this model does not require release of endogenous 5-HT and would explain why GR-127935 did not always have an effect on the "diving response". The autoreceptor model of 5-HT_{1D} receptor function requires release of 5-HT from a serotonergic terminal to stimulate both pre- and postsynaptic 5-HT receptors. One method to investigate these hypotheses would be to pretreat a group of rabbits with the serotonergic neurotoxin 5,7-dihydroxytryptamine before administering a 5-HT_{1D} receptor agonist or antagonist. If the inhibitory effect of the 5-HT_{1D} receptor agonist was unaffected or increased, but the excitatory effect of the 5-HT_{1D} receptor antagonist was abolished this would provide evidence that the drugs were acting on 5-HT_{1D} heteroreceptors located on a non-serotonergic neurone. The role of 5-HT_{1D} receptors could also be investigated by depleting serotonergic neurones of 5-HT using the 5-HT synthesis inhibitor, parachlorophenylalanine. This would also be predicted to abolish the effect of 5-HT_{1D} receptor antagonists but not 5-HT_{1D} receptor agonists.

Evidence that 5-HT_3 receptors play a role in the reflex activation of cardiac vagal motoneurones in rabbits.

The highest densities of 5-HT_3 receptors in the CNS are located in discrete nuclei of the lower brain stem, including the dorsal vagal motonucleus (DVMN), nucleus tractus solitarius (NTS) and spinal trigeminal nucleus (Waeber et al., 1988; Hamon et al., 1989; Pratt et al., 1990). Within the NTS the 5-HT_3 receptors are thought to be located on vagal afferent terminals (Pratt & Bowery, 1989), since unilateral nodose ganglionectomy reduces the number of 5-HT_3 binding sites in the ipsilateral NTS by 50%. It has been demonstrated that 5-HT_3 receptors are involved in the modulation of neurotransmitter release both from dopaminergic (excitatory; Blandina et al., 1988) and cholinergic neurones (inhibitory; Barnes et al., 1989).

Effects of the 5-HT_3 receptor antagonist, granisetron on resting variables.

Administration of the highly selective 5-HT_3 receptor antagonist granisetron i.c. (Sanger & Nelson, 1989) had no significant effects on resting R-R interval, mean arterial blood pressure, phrenic burst rate or baseline renal nerve activity. Since the dose of granisetron administered i.c. did cause significant changes to the "diving response", it is concluded that the dose of granisetron was reaching brain stem 5-HT_3 receptors, but that there was little activity at these 5-HT receptors when the rabbit was at rest.

Effects of the 5-HT_3 receptor antagonist, granisetron on the "diving response".

The smoke evoked increase in R-R interval and the apnoea were both inhibited and the hypertension and sympathoexcitation were unchanged following administration of granisetron (i.c.). Administration of granisetron i.v. caused no changes to either baseline or reflex variables, suggesting that the 5-HT_3 receptors are central. It is therefore concluded that central 5-HT_3 receptors are excitatory to the smoke response bradycardia and apnoea. The observation that neither the renal sympathoexcitation nor the reflex increase in mean arterial blood pressure are altered by administration of granisetron (i.c.) suggests that the excitatory effect of 5-HT_3 receptors on the reflex is not occurring at the primary afferent terminal since this would be predicted to inhibit all components of the response.

Effects of other ligands on reflex activation of cardiac vagal motoneurones in rabbits.

Effects of the D_2 receptor antagonist, sulpiride on resting variables.

In addition to 5-HT_{1A} receptor agonist activity (Hoyer & Fozard, 1991), buspirone also has similar affinity for D_2 dopaminergic receptors, where it is an antagonist (Piercy et al., 1994). To investigate the possibility that some of the effects of buspirone may be mediated via D_2 receptor antagonism, the D_2 receptor antagonist sulpiride was administered i.c. Sulpiride caused a significantly shortened baseline R-R interval, accompanied by a highly significant increase in baseline MAP and tended to increase RNA. Since all of these effects are different to those of buspirone, it is concluded that the mechanism of action of buspirone on the resting variables was not mediated through D_2 receptor antagonism.

Effects of the D_2 receptor antagonist, sulpiride on the "diving response".

Administration of sulpiride (i.c.) had small effects on the "diving response", it tended to increase the hypertension and inhibit the apnoea. Since all of these effects are different to those of buspirone, it is concluded that buspirone did not act on the "diving response" through D_2 receptor antagonism.

Evidence that 5-HT_7 receptors do not modulate the "diving response".

It has been demonstration that 8-OH-DPAT has affinity and a partial agonist activity at 5-HT_7 receptors (Lovenberg et al., 1993). This suggests that these receptors may also be involved. The failure to see any effects of mesulergine, which has been demonstrated to be a competitive antagonist at 5-HT_7 receptors (Lovenberg et al., 1993), on the resting variables or the "diving response" indicates that 5-HT_7 receptors are not activated. Furthermore, as mesulergine is also an antagonist at 5-HT_2 receptors, this suggests that these are also not activated during the "diving response".

A species difference exists between rabbits and rats.

Previous research has found that the cardiopulmonary chemoreflex triggered by PBG in the rat is inhibited by buspirone (Bogle et al., 1990). In contrast, the diving response bradycardia in the rabbit is potentiated by buspirone (Futuro-Neto et al., 1993). This difference could reflect either a species variation or a difference in the effect of buspirone on the 2 reflexes. In the present study, administration of buspirone to the rabbit caused potentiation of both the cardiopulmonary chemoreflex and "diving response" bradycardias. In contrast, administration of buspirone to the rat caused inhibition of the "diving response" bradycardia. This suggested that there may be a species difference in the modulation of the "diving response" and pulmonary C-fibre reflex by 5-HT_{1A} receptors.

Effects of 5-HT_{1A} ligands in anaesthetised rats.

Effects of 5-HT_{1A} receptor agonists on resting variables.

It has been demonstrated that administration of 8-OH-DPAT causes a bradycardia and hypotension (Gradin et al., 1985; Fozard et al., 1987) and microinjection of 5-HT_{1A} receptor agonists into the vicinity of cardiac vagal motoneurones in the dorsal vagal motonucleus caused increased baseline vagal tone and respiratory drive (Sporton et al., 1989) in anaesthetised rats. In the present study, buspirone (i.c.) or 8-OH-DPAT (i.c.) caused a significant increase in resting R-R interval and increased phrenic burst rate. In addition 8-OH-DPAT also caused a small and significant hypotension. This may be due to an action in the rostral ventrolateral medulla since iontophoretic application of 5-HT or 5-HT_{1A} receptor agonists to neurones in this region inhibits their on-going activity (Wang & Lovick, 1992). Pretreatment with a 5-HT_{1A} antagonist, WAY-100802 (i.c.) significantly reduced the effects of (+)8-OH-DPAT on resting R-R interval, phrenic burst rate and resting mean arterial blood pressure, supporting the view that these effects were mediated through the 5-HT_{1A} agonist activity of this drug. Furthermore, in the present study the rats were always pretreated with the β-adrenoceptor antagonist, atenolol (i.v.) so the increase in resting R-R interval can be attributed to increased basal cardiac vagal motoneurone activity rather than a cardiac sympathoinhibition.

Effects of 5-HT$_{1A}$ receptor antagonists on resting variables.

In the present study, administration (i.c.) of the 5-HT$_{1A}$ antagonists, WAY-100635, WAY-100802 (Cliffe et al., 1994) and (-)pindolol did not alter resting R-R interval, but caused a significant reduction of phrenic burst rate. In addition, WAY-100635 also caused a significant hypotension. It has been demonstrated in the rat that WAY-100635 has a much greater affinity for 5-HT$_{1A}$ receptors over other receptor types and that the drug has no partial agonist activity at either 5-HT$_{1A}$ somatodendritic autoreceptors or 5-HT$_{1A}$ heteroreceptors (Forster et al., 1995). It is therefore concluded that the effects of these drugs on phrenic burst rate were due to an antagonist action at 5-HT$_{1A}$ receptors.

Effects of 5-HT$_{1A}$ receptor agonists on the "diving response".

In the present study, administration of the 5-HT$_{1A}$ agonists buspirone and (+)8-OH-DPAT both inhibited the reflex bradycardia and apnoea of the "diving response". Since both drugs virtually abolished the apnoea component of the "diving response" it is very likely that a substantial part of the inhibition of the bradycardia can be attributed to lung stretch receptor activity (Angell-James & Daly, 1978) and inspiratory respiratory gating (Gilbey et al., 1984) making the cardiac vagal motoneurones refractory to the excitatory input of the "diving response". The hypothesis that the 5-HT$_{1A}$ agonists are inhibiting the "diving response" bradycardia by attenuating the apnoea could be tested by artificially hyperventilating a group of rats to abolish phrenic nerve activity before repeating the "diving response" experiments in the absence of respiratory activity. It is unlikely that (+)8-OH-DPAT is inhibiting the response in the rat by the same mechanism as in the rabbit because the affinity of 8-OH-DPAT for 5-HT$_{1B}$ receptors is low ($pK_D = 4.2$) and that of buspirone even lower ($pK_D = 3.9$; see Hoyer & Fozard, 1991).

Effects of 5-HT$_{1A}$ receptor antagonists on the "diving response".

A previous study demonstrated that administration (i.c.) of a variety of 5-HT$_{1A}$ receptor antagonists caused inhibition of the reflex bradycardia of the pulmonary C-fibre reflex (Bogle et al., 1990). In the present experiments, administration (i.c.) of the 5-HT$_{1A}$

receptor antagonists WAY-100635 and WAY-100802 had no significant effect on the "diving response" reflex bradycardia or hypertension, although both drugs significantly prolonged the apnoea. In contrast, (-)pindolol did not significantly alter the apnoea duration, but did inhibit the reflex bradycardia and the hypertension. It could be argued that WAY-100635 and WAY-100802 were inhibiting the activation of cardiac vagal motoneurones, but that because they also potentiated the duration of the apnoea, the chemoreceptor drive was greater at the end of the "diving response", resulting in no overall change in the peak R-R interval increase. By measuring the change in R-R interval at 3 s after the upper airways had been stimulated, the effect of an increase in apnoea length increasing the chemoreceptor drive at the end of the response is avoided. A baseline "diving response" apnoea was 6 ± 1 s in duration in the WAY-100635 group (n=4), so the bradycardia was well developed at this point in the rat (14 ± 4 ms). It was found that the increase in R-R interval 3 s after stimulating the upper airways was not changed (0 ± 1 ms after 5 min) by administration of WAY-100635 (i.c.), demonstrating that the lack of change of the bradycardia was not due to the apnoea duration changing. In light of the observation that WAY-100635, a well characterised 5-HT_{1A} antagonist had no effect on the "diving response" bradycardia in the halothane-urethane anaesthetised rat, the inhibition of the bradycardia by (-)pindolol cannot be attributed to 5-HT_{1A} antagonism, but is more likely to be due to β-adrenoceptor antagonism or a local anaesthetic effect.

The effects of (+)8-OH-DPAT are mediated by 5-HT_{1A} receptors.

It was demonstrated that the effects of (+)8-OH-DPAT are mediated through 5-HT_{1A} receptor agonism, since pretreatment with a 5-HT_{1A} receptor antagonist, WAY-100802 (i.c.), inhibited the effects of (+)8-OH-DPAT on resting R-R interval, mean arterial blood pressure and phrenic burst rate. In addition, WAY-100802 blocked the (-)8-OH-DPAT mediated inhibition of the "diving response" reflex bradycardia and apnoea, and the reduction of the reflex hypertension produced by (+)8-OH-DPAT alone was changed into a potentiation of the hypertension if (+)8-OH-DPAT was administered in the presence of WAY-100802. This provides further evidence that the effects of (+)8-OH-DPAT were mediated through activation of 5-HT_{1A} receptors.

Effects of a 5-HT_3 ligand in rats.

It has been demonstrated that the highest densities of 5-HT_3 receptors in the rat brain are located in discrete nuclei of the lower brain stem, particularly in the nucleus tractus solitarius (NTS) as well as the dorsal vagal motonucleus and spinal trigeminal nucleus (Pratt et al., 1990). Within the NTS of the rat the 5-HT_3 receptors are thought to be located on vagal afferent terminals (Pratt & Bowery, 1989), since unilateral nodose ganglionectomy reduces the number of 5-HT_3 binding sites in the ipsilateral NTS by 50%.

Granisetron does not change either the resting variables or the "diving response" in anaesthetised rats.

A previous study demonstrated that administration (i.c.) of the 5-HT_3 receptor antagonist ICS 205-930 had no effect on either the resting variables or the pulmonary C-fibre reflex in the anaesthetised rat (Bogle et al., 1990). In the present study the 5-HT_3 receptor antagonist granisetron was administered (i.c.) at a dose that caused a large inhibition of the "diving response" in the rabbit. This drug did not change resting R-R interval, phrenic burst rate or mean arterial blood pressure, nor did it alter the "diving response". It could be argued that the dose used (20 $\mu g\ kg^{-1}$) was inadequate, however, it has been demonstrated in the rat that granisetron can inhibit the chemoreflex produced by 2-methyl-5-HT (i.v.) with an ID50 of 0.17 $\mu g\ kg^{-1}$ (Eglen et al., 1994) and granisetron has been shown to have an anxiolytic effect in rats at a dose of 10 $\mu g\ kg^{-1}$ i.p.(Nevins & Anthony, 1994). It is concluded that a species difference exists between rabbits and rats with respect to their response to 5-HT_3 receptor antagonists. This may reflect a low level of activity at these receptors in the rat, both at rest and during the "diving response", so administration of an antagonist does not cause any changes.

Conclusions.

Data obtained from rabbits.

1. There is evidence that (+)8-OH-DPAT and buspirone administered i.c. are activating 5-HT_{1A} receptors to increase the basal activity of cardiac vagal motoneurones and respiratory rate and cause a sympathoinhibition since these effects are all blocked by pretreatment with a selective 5-HT_{1A} antagonist, WAY-100635.

2. It has been demonstrated that the potentiation of the "diving response" bradycardia elicited by administration of buspirone (i.c.) is due to activation 5-HT_{1A} receptors as it can be abolished by pretreatment with WAY-100635. This effect is not dependent on the secondary arterial chemoreceptor stimulation that occurs during the "diving response" since it is still present when the animals are kept hyperoxic or artificially ventilated. In addition, the effects of buspirone are not specific to the bradycardia evoked by upper airway stimulation since that evoked by stimulation of pulmonary C-fibre afferents is also potentiated.

3. It has been demonstrated that the inhibition of the "diving response" bradycardia elicited by administration of (+)8-OH-DPAT (i.c.) is due to activation of 5-HT_{1D} receptors since the effect is blocked by pretreatment with the 5-HT_{1D} receptor antagonist, GR-127935, and is mimicked by sumatriptan, a 5-HT_{1D} receptor agonist.

4. There is evidence that (+)8-OH-DPAT (i.c.) is not inhibiting the "diving response" bradycardia through changes in baseline respiratory rate or evoked apnoea duration since these changes are blocked by pretreatment with WAY-100635, without significantly altering the inhibition of the bradycardia.

5. It has been demonstrated that administration of granisetron (i.c.) inhibits the "diving response" bradycardia and apnoea duration, but that granisetron (i.v.) has no effect, evidence that central 5-HT_3 receptors play an excitatory role in these aspects of the "diving response".

Data obtained from rats.

6. There is evidence that buspirone and (+)8-OH-DPAT are activating cardiac vagal motoneurones and increasing phrenic burst rate through activation of 5-HT_{1A} receptors, since the effects of (+)8-OH-DPAT are inhibited by pretreatment with a 5-HT_{1A} receptor antagonist, WAY-100802.

7. A species difference exists between rabbits and rats. Buspirone inhibits the "diving response" bradycardia in rats and granisetron does not affect it.

8. Both buspirone (i.c.) and (+)8-OH-DPAT (i.c.) inhibit the "diving response" bradycardia and apnoea. The effect of (+)8-OH-DPAT is blocked by pretreatment with WAY-100802, evidence that the effects are due to the 5-HT_{1A} receptor agonist activity of these drugs. From the experiments performed so far it is not possible to rule out inhibition of the apnoea duration as the mechanism by which these drugs are inhibiting the bradycardia.

Further experiments to perform.

1. To establish whether the effects of buspirone and (+)8-OH-DPAT on the "diving response" in the rat are due to their effects on the apnoea duration or a more direct effect on the reflex activation of cardiac vagal motoneurones, a group of rats could be artificially hyperventilated to abolish phrenic nerve activity before the "diving response" experiment is performed. This would exclude the effects of alterations in respiratory drive on the response, but would have the disadvantage that activation of lung stretch receptors would inhibit the "diving response" bradycardia, although this could be minimised by creating a pneumothorax and using a fast rate and low volume to ventilate the lungs.

2. An alternative would be to try low intensity electrical stimulation of the superior laryngeal nerve to elicit an apnoea without causing a bradycardia just before the smoke

challenge is delivered. This would allow the apnoea length to be standardised excluding the effects of changes in apnoea length and would not be associated with activation of lung stretch receptors.

3. To establish whether the 5-HT_{1D} receptors inhibiting the "diving response" in rabbits are autoreceptors inhibiting release of 5-HT from serotonergic neurones, or heteroreceptors inhibiting release of transmitter from, for instance cholinergic or glutaminergic neurones, a group of rabbits could be pretreated with the serotonergic neurotoxin, 5,7- dihydroxytryptamine , or the 5-HT synthesis inhibitor, parachlorophenylalanine. If administration of a 5-HT_{1D} receptor agonist still inhibited the "diving response" bradycardia this would provide evidence that the receptors responsible are heteroreceptors located on non-serotonergic neurones.

4. It is known that the 5-HT_{1D} receptor is not represented in the rat to a great extent, where the 5-HT_{1B} receptor appears to be localised in its place (see Hoyer et al., 1994). At present there are no selective 5-HT_{1B} receptor agonists, but one strategy to investigate the role of these receptors in the rat would be to administer a less selective drug, for instance, 5-carboxamidotryptamine in the presence of WAY-100635.

5. It has already been established that the bradycardias evoked by pulmonary C-fibre stimulation and the "diving response" are potentiated by buspirone in the rabbit. To discover whether or not this 5-HT_{1A} receptor ligand has the same effect on other reflex bradycardias, the arterial chemoreflex and baroreflex could be studied, using sodium cyanide injection into the external carotid artery or inflating a balloon catheter in the carotid sinus region respectively. Again, it would be necessary to exclude the effects of altered respiratory drive, for instance by hyperventilating as described above.

6. There are many sites within the brain stem at which 5-HT receptor ligands applied intracisternally could be acting. The nucleus tractus solitarius, nucleus ambiguus and dorsal vagal motonucleus are all sites of great importance in the reflex activation of

cardiac vagal motoneurones known to receive serotonergic innervation and contain high densities of 5-HT receptors. The rostral ventrolateral medulla, raphe nuclei and anterior hypothalamus are all of great importance in the control of sympathetic outflow and also contain many serotonergic neurones and receptors. Microinjection of drugs into these sites (Sporton et al., 1991) would provide one approach to investigating their role in these reflexes. However, the technique is not ideal since to produce a detectable change to the "diving response" bradycardia it would probably be necessary to microinject a drug bilaterally into, for instance the nucleus ambiguus. It would be technically demanding to accurately microinject a drug into the same part of the nucleus bilaterally.

7. A different approach to localise more specifically the site of action of the 5-HT receptor ligands would be to make recordings of neurones, for instance in the nucleus tractus solitarius, which could be identified as receiving cardiorespiratory afferents (see Jordan & Spyer, 1987), or in the nucleus ambiguus, where preganglionic cardiac vagal motoneurones could be identified antidromically (Jordan et al., 1982). Using multi-barelled glass microelectrode would allow ionophoretic application of selective 5-HT ligands to functionally identified brain stem neurones (Wang et al., 1994) and so the effects of these ligands on reflexly evoked and on-going activity from nasopharyngeal, pulmonary, baroreceptor and chemoreceptor afferents could be investigated. The main problem with this technique is the difficulty of holding onto an identified neurone during the large changes in blood pressure associated with these reflexes.

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Appendices

Appendix 5.1; Spike2 script written to analyse R-R interval changes.

```
numvar 8
var bas res fes pea ana sbd jmd pbd'                 sets variables
normal;clear
view 2 "R-R interval plot";window 0 0 100 95;on title
view 1;window 0 0 100 95;on labels
repeat
SCATTER'                                             runs routines
MARK
SETUP
CALC3
FIVE
CALC5
PEAK

moveto 20 20'                                        performs calculations after the
printto 1'                                           R-R intervals have been
sbd:=res-bas*1000'                                   measured.
sbd:=round(sbd)'                                     rounds 3s value to nearest ms
print 1 "3 sec R-R change is = %d ms" sbd'           prints 3s value
jmd:=fes-bas*1000'
jmd:=round(jmd)'                                     rounds 5s value to nearest ms
print 1 "5 sec R-R change is =%d ms" jmd'            prints 5s value
pbd:=pea-bas*1000'
pbd:=round(pbd)'                                     rounds peak value to ms
print 1 "peak R-R change is =%d ms" pbd'             prints peak change
END'                                                 stops programme

PROC MARK'                                           to locate stimulus
view 3;window 0 0 100 95
off all;on 7'                                        display marker channel
draw 0 maxtime
cursors 1
print 0 "Find marker"
interact'                                            locates mark
print 1 "%d" c1'                                     prints position of mark in file
ana:=c1'                                             sets variable as marker
delay 1'                                             position
RETURN

PROC SCATTER'                                        to draw a point for each R wave
newevent 0
newevent 1 16000'                                    set minimum sized buffer event
newevent 6 3 0 maxtime'                              load buffer
setaxes 2'                                           establish view 2 for drawing
view 2'                                              make it current view
yrange 1 0.1 0.8'                                    set scale in s
draw 0 300'                                          draw first 300 s
print 0 "drawing scatter plot for %d events" event
for i:=2 event'                                      go from 2nd to final event
   x:=event[i]-event[i-1]'                           set variable as event no.
   mover 1 event[i] x'                               move to this event
   drawr 1 event[i] x'                               draw this event
   next i'                                           go to next event
RETURN
```

PROC SETUP'	measure baseline R-R interval
view 2	
cursors 1'	select 1 vertical cursor
setc 1 ana'	position it over mark
print 0 "scanning in"	
draw ana-2 ana+30'	draw 2s before to 30s after mark
for i:=2 event'	go from 2nd to final event
x:=event[i]-event[i-1]'	set variable as event no.
mover 1 event[i] x'	move to this event
drawr 1 event[i] x'	draw this event
next i'	go to next event
hcursor 1'	select horizontal cursor
interact'	find baseline R-R interval
setc 1 ana+3'	moves vertical cursor on 3s
bas:=hc'	sets baseline variable
print 0 "baseline is %d" bas'	prints baseline variable
RETURN	
PROC CALC3'	measure 3s R-R interval
print 0 "3s response"	
interact'	find 3s R-R interval
res:=hc'	sets 3s variable
RETURN	
PROC FIVE'	move vertical cursor to 5s point
setc 1 c1+2	
RETURN	
PROC CALC5'	measures 5s R-R interval
print 0 "5s response"	
hcursor 1	
interact'	find 5s R-R interval
fes:=hc'	sets 5s variable
RETURN	
PROC PEAK'	measures peak R-R interval
print 0 "select peak response"	
hcursor 1	
interact'	find peak R-R interval
pea:=hc'	sets peak R-R variable
RETURN	

Appendix 5.2; Spike2 script written to measure changes in blood pressure.

numvar 13	
var ana bsy bdi tsy tdi fsy fdi'	sets variables
var psy pdi bap tap fap sbd	
normal;clear;view 1;window 0 0 100 95	
yrange 1 0 200	
repeat	
MARK'	runs routines
SETUP	
SETDI	
THREE	
TDIA	
FIVE	

```
FDIA
PEAK
PDIA
ROUN
CALC
END

PROC MARK'                                  locates mark
view 2;window 0 0 100 95
off all;on 7                                displays marker channel
draw 0 maxtime'                             draws channel
cursors 1'                                  vertical cursor on
print 0 "find marker"
interact'                                   locates mark
print 0 "%d" c1                             displays mark position in file
ana:=c1                                     sets mark variable
RETURN

PROC SETUP'                                 measures baseline systolic BP
view 1
off all;on 1'                               displays BP channel
yrange 1 0 200
cursors 1'                                  vertical cursor on
setc 1 ana'                                 positions cursor over mark
print 0 "scanning in"
draw ana-5 ana+60'                          draw 5s before to 60s after mark
hcursor 1'                                  horizontal cursor on
printto 0
print "Choose baseline systolic BP"
interact'                                   measure systolic BP
bsy:=hc'                                    set baseline systolic BP variable
RETURN

PROC SETDI'                                 measures baseline diastolic BP
hcursor 1'                                  horizontal cursor on
printto 0
print "Choose baseline diastolic BP"
interact'                                   measures baseline diastolic BP
bdi:=hc'                                    set baseline diastolic BP variable
RETURN

PROC THREE'                                 measures 3s systolic BP
print 0 "3s response"
setc 1 ana+3'                               move cursor on 3s
hcursor 1'                                  horizontal cursor on
print 0 "Choose 3s systolic BP"
interact'                                   measures systolic 3s BP
tsy:=hc'                                    set 3s systolic BP variable
RETURN

PROC TDIA'                                  measure 3s diastolic BP
hcursor 1
print 0 "Choose 3s diastolic BP"
interact'
tdi:=hc'                                    set 3s diastolic BP variable
RETURN

PROC FIVE'                                  measures 5s systolic BP
setc 1 ana+5'                               move vertical cursor on 5s
hcursor 1
```

```
print 0 "Choose 5s systolic BP"
interact
fsy:=hc'                                        set 5s systolic BP variable
RETURN

PROC FDIA'                                      measures 5s diastolic BP
hcursor 1
print 0 "Choose 5s diastolic BP"
interact
fdi:=hc'                                        set 5s diastolic variable
RETURN

PROC PEAK'                                      measures peak systolic BP
hcursor 1
print 0 "Choose peak systolic BP"
interact
psy:=hc'                                        sets peak systolic variable
RETURN

PROC PDIA'                                      measures peak diastolic BP
hcursor 1
print 0 "Choose peak diastolic BP"
interact
pdi:=hc'                                        sets peak diastolic variable
RETURN

PROC ROUN'                                      rounds values to nearest mmHg
bsy:=round(bsy)
bdi:=round(bdi)
tsy:=round(tsy)
tdi:=round(tdi)
fsy:=round(fsy)
fdi:=round(fdi)
psy:=round(psy)
pdi:=round(pdi)
bap:=round(bap)
tap:=round(tap)
fap:=round(fap)
sbd:=round(sbd)
RETURN

PROC CALC'                                      displays measured BP values
moveto 10 10
printto 1
print "        Baseline systolic  =%d " bsy'    baseline BP
print"                 diastolic =%d mmHg" bdi
print"     3s response systolic  =%d " tsy'      3s BP
print"                 diastolic =%d mmHg" tdi
print"     5s response systolic  =%d " fsy'      5s BP
print"                 diastolic =%d mmHg" fdi
print"   peak response systolic  =%d " psy'      peak BP
print"                 diastolic =%d mmHg" pdi
moveto 70 10
bap:=bdi+((bsy-bdi)/3)'                         calculate baseline mean BP
bap:=round(bap)
print "Base MAP= %d" bap'                       baseline MAP
tap:=tdi+((tsy-tdi)/3)'                         calculate 3s MAP
tap:=round(tap)
print"   3s MAP=%d" tap'                        3s MAP
fap:=fdi+((fsy-fdi)/3)'                         calculate 5s MAP
```

```
fap:=round(fap)
print"    5s MAP=%d" fap'                     5s MAP
sbd:=pdi+((psi-pdi)/3)'                       calculate peak MAP
sbd:=round(sbd)
print "peak MAP=%d" sbd'                      peak MAP
print "P cg MAP=%d" sbd-bap'                  peak change MAP
RETURN
```

Appendix 5.3; Spike2 script written to measure integrated renal nerve activity.

```
numvar 4
var bas res ans ana'                          sets variables
MARK'                                         runs routines
MEAS

PROC MARK'                                    locates marker
view 2;window 0 0 100 95
normal
off all;on 7'                                 displays marker channel
draw 0 maxtime
cursors 1'                                    vertical cursor on
print 0 "find marker"
interact'                                     locate mark
print 0 "%d" c1'                              displays position of mark
ana:=c1'                                      set mark variable
RETURN

PROC MEAS'                                    measures baseline + response
view 1;window 0 0 100 95'                     renal nerve activity
off all
on 8'                                         displays integrator output
yrange 8 -0.2 10
draw 0 maxtime
count 8 ana ana+30 b'                         measures 30s after mark
count 8 ana-30 ana a'                         measures 30s before mark
moveto 20 20
print 1 "baseline= %d" a
print 1 "response=%d" b
ans:=(b/a)*100'                               calculates % change activity
ans:=round(ans)
print 1 "response RNA= %d % the size of baseline RNA." ans
hcursor 0'                                    horizontal cursor off
END
```

Appendix 5.4; Spike2 script written to measure apnoea duration from raw PNA.

```
numvar 2
var sta fin'                                  set variables
view 1;window 0 0 100 95
off all
on 6'                                         display PNA channel
yrange 6 -5 5
draw 0 maxtime
cursors 2 1'                                  set 2 vertical cursors in view 1
```

```
print 0 "Select apnoea period."
interact'                                  find start and end of apnoea
sta:=c1
fin:=c2
sta:=round(sta)
fin:=round(fin)
moveto 10 10
print 1 "Apnoea duration is %d s." fin-sta'    calculate apnoea duration
END
```

Appendix 5.5

Table 5.1 Resting baseline values, before drug.

Drug	Dose and route	n	Baseline MAP (mmHg)	Baseline R-R interval (ms)	Baseline respiratory rate (breaths min^{-1})
Saline (Vehicle)	20 μl i.c.	5	55 ± 3	243 ± 8	50 ± 5
Buspirone	200 μg kg^{-1} i.c.	5	64 ±6	259 ±13	56 ± 5
(+)8-OH-DPAT	25 μg kg^{-1} i.c.	5	52 ± 7	262 ±11	55 ± 3
WAY-100635	100 μg kg^{-1} i.c.	5	55 ± 5	265 ± 7	53 ± 4
WAY-100635	100 μg kg^{-1} i.v.	5	61 ± 5	262 ± 4	56 ± 3
nn-DP-5-CT	50 μg kg^{-1} i.c.	5	61 ± 5	266 ± 11	58 ± 5
Granisetron	20 μg kg^{-1} i.c.	5	60 ± 6	259 ± 10	71 ± 7
Granisetron	20 μg kg^{-1} i.v.	5	49 ± 6	257 ± 11	55 ± 8
Buspirone (after WAY-100635)	200 μg kg^{-1} i.c. (100 μg kg^{-1} i.v.)	5	63 ± 6	256 ± 6	55 ± 2
Sumatriptan (after WAY-100635)	200 μg kg^{-1} i.c. (100 μg kg^{-1} i.v.)	5	49 ± 6	250 ± 8	52 ± 3

Drug	Dose and route	n	Baseline MAP (mmHg)	Baseline R-R interval (ms)	Baseline respiratory rate (breaths min^{-1})
(+)8-OH-DPAT (after GR-127935)	25 μg kg^{-1} i.c. (100 μg kg^{-1} i.v.)	4	46 ± 2	265 ± 6	66 ± 13
(+)8-OH-DPAT (after WAY-100635)	25 μg kg^{-1} i.c. (100 μg kg^{-1} i.v.)	4	69 ± 4	269 ± 11	59 ± 3
Acidified saline (Vehicle)	20 μl i.c.	5	48 ± 5	273 ± 5	38 ± 3
Sulpiride	200 μg kg^{-1} i.c.	4	39 ± 2	260 ± 6	46 ± 5
(-)pindolol	100 μg kg^{-1} i.c.	5	41 ± 2	265 ± 3	54 ± 8
Distilled water (Vehicle)	20 μl i.c.	4	65 ± 3	264 ± 6	67 ± 3
Mesulergine	200 μg kg^{-1} i.c.	4	58 ± 4	283 ± 43	47 ± 8
GR-127935	20 μg kg^{-1} i.c.	5	65 ± 5	252 ± 7	71 ± 7
GR-127935	100 μg kg^{-1} i.v.	4	55 ± 6	273 ± 11	66 ± 14
Atenolol	1 mg kg^{-1} i.v.	5	76 ± 4	216 ± 4	68 ± 15

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Resting baseline values after drug.

Table 5.2 **Saline 20 μl i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	55 ± 3	53 ± 3 **(2 ± 2)**	51 ± 4 **(-4 ± 2)**	50 ± 4 **(-5 ± 3)**	51 ± 5 **(-4 ± 3)**
R-R interval (ms)	243 ± 8	244 ± 8 **(1 ± 1)**	245 ± 8 **(2 ± 3)**	243 ± 7 **(0 ± 4)**	245 ± 8 **(2 ± 5)**
Phrenic burst rate(bursts min^{-1})	50 ± 5	51 ± 5 **(1 ± 1)**	51 ± 5 **(2 ± 2)**	52 ± 5 **(2 ± 2)**	54 ± 6 **(4 ± 3)**
RNA (%)	100	90 ± 5 **(-9 ± 5)**	87 ± 4 **(-12 ± 4)**	93 ± 5 **(-7 ± 5)**	101 ± 5 **(1 ± 5)**

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Table 5.3 **Buspirone 200 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	64 ± 6	52 ± 6 **(-12 ± 4*)**	53 ± 5 **(-11 ± 1)**	56 ± 6 **(-9 ± 2)**	57 ± 6 **(-8 ± 2)**
R-R interval (ms)	259 ± 13	282 ± 11* **(23 ± 8*)**	281 ± 11* **(22 ± 3*)**	277 ± 12* **(18 ± 4*)**	273 ± 13 **(14 ± 4)**
Phrenic burst rate(bursts min^{-1})	56 ± 5	73 ± 4 **(25 ± 13*)**	83 ± 4** **(35 ± 11**)**	98 ± 2** **(50 ± 11**)**	85 ± 6** **(37 ± 10**)**
RNA (%)	100	63 ± 21 **(-37 ± 21)**	64 ± 25 **(-35 ± 25)**	44 ± 16* **(-56 ± 17*)**	44 ± 19****(-56 ±19**)**

Statistical comparison = saline : buspirone

Table 5.4 **(+)8-OH-DPAT 25 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	52 ± 7	47 ± 5 **(-5 ± 3)**	53 ± 2 **(2 ± 6)**	51 ± 4 **(0 ± 6)**	48 ± 4 **(-4 ± 4)**
R-R interval (ms)	262 ± 11	265 ± 9 **(3 ± 3)**	279 ± 9** **(17 ± 5)**	284 ± 7****(22 ± 5**)**	277 ± 6****(15 ± 6**
Phrenic burst rate(bursts min^{-1})	55 ± 3	110 ± 10****(55 ± 10**)**	146 ± 26****(91 ± 24**)**	147 ± 25****(92 ± 24**)**	141 ± 22****(87 ± 21**
RNA (%)	100	59 ± 12****(-41 ± 12**)**	63 ± 12***(-37 ± 12*)**	72 ± 11 **(-28 ± 11*)**	76 ± 10***(-24 ± 10**

Statistical comparison = saline : (+)8-OH-DPAT

Table 5.5 **WAY-100635 100 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	55 ± 5	56 ± 5 **(-1 ± 4)**	52 ± 4 **(-3 ± 2)**	54 ± 6 **(-3 ± 2)**	54 ± 7 **(-1 ± 3)**
R-R interval (ms)	265 ± 7	278 ± 12* **(13 ± 7)**	273 ± 13 **(8 ± 6)**	270 ± 13 **(5 ± 7)**	267 ± 14 **(2 ± 8)**
Phrenic burst rate(bursts min^{-1})	53 ± 4	51 ± 11 **(-2 ± 9)**	50 ± 8 **(-2 ± 6)**	50 ± 4 **(-2 ± 2)**	52 ± 4 **(-1 ± 2)**
RNA (%)	100	107 ± 2 **(7 ± 2)**	87 ± 15 **(-13 ± 15)**	91 ± 11 **(-9 ± 11)**	106 ± 15 **(6 ± 15)**

Statistical comparison = saline : WAY-100635

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Table 5.6 **WAY-100635 100 μg kg^{-1} i.v. n=5.**

Time after drug (min)	-5	5	15
MAP (mmHg)	61 ± 5	68 ± 5* **(7 ± 1)**	60 ± 4 **(-1 ± 3)**
R-R interval (ms)	262 ± 4	263 ± 4 **(1 ± 1)**	260 ± 4 **(-2 ± 3)**
Phrenic burst rate(bursts min^{-1})	56 ± 3	54 ± 2 **(-2 ± 2)**	57 ± 4 **(2 ± 2)**
RNA (%)	100	102 ± 1 **(3 ± 1*)**	99 ± 1 **(-2 ± 1*)**

Statistical comparison = saline : WAY-100635

Table 5.7 **nn-DP-5-CT 50 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	61 ± 5	54 ± 4 **(-7 ± 2)**	52 ± 4 **(-9 ± 3)**	51 ± 5 **(-10 ± 4)**	58 ± 8 (-4 ± 4)
R-R interval (ms)	266 ± 11	270 ± 13 **(4 ± 3)**	274 ± 14 **(8 ± 5)**	286 ± 17***(20 ± 9*)**	289 ± 17* (23 ± 9*)
Phrenic burst rate(bursts min^{-1})	58 ± 5	71 ± 4 **(13 ± 7)**	88 ± 12* **(30 ± 15)**	110 ± 15****(51 ± 19**)**	138 ± 22**(80 ± 25**)
RNA (%)	100	95 ± 6 **(-5 ± 6)**	61 ± 14* **(-39 ± 14*)**	57 ± 12****(-43 ± 12**)**	60 ± 18**(-40 ± 18**)

Statistical comparison = saline : DP-5-CT

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Table 5.8 **Granisetron 20 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	60 ± 6	52 ± 5 **(-8 ± 3)**	48 ± 3 **(-12 ± 4)**	47 ± 3 **(-13 ± 5)**	46 ± 3 **(-13 ± 4)**
R-R interval (ms)	259 ± 10	255 ± 9 **(-4 ± 2)**	255 ± 9 **(-4 ± 2)**	254 ± 9 **(-5 ± 2)**	253 ± 10 **(-6 ± 2)**
Phrenic burst rate(bursts min^{-1})	71 ± 7	69 ± 10 **(0 ± 3)**	73 ± 11 **(1 ± 5)**	74 ± 11 **(2 ± 5)**	72 ± 10 **(1 ± 4)**
RNA (%)	100	109 ± 15 **(9 ± 15)**	112 ± 18 **(32 ± 27*)**	114 ± 16 **(35 ± 25*)**	112 ± 16 **(12 ± 16)**

Statistical comparison = saline : granisetron

Table 5.9 **Granisetron 20 μg kg^{-1} i.v. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	49 ± 6	47 ± 5 **(-3 ± 1)**	47 ± 4 **(-2 ± 4)**	45 ± 3 **(-4 ± 3)**	45 ± 3 **(-5 ± 4)**
R-R interval (ms)	257 ± 11	257 ± 11 **(0 ± 0)**	256 ± 10 **(-1 ± 2)**	253 ± 10 **(-4 ± 2)**	254 ± 10 **(-3 ± 3)**
Phrenic burst rate(bursts min^{-1})	55 ± 8	53 ± 6 **(-4 ± 3)**	53 ± 7 **(-1 ± 2)**	55 ± 9 **(1 ± 1)**	54 ± 8 **(-1 ± 1)**
RNA (%)	100	94 ± 7 **(-6 ± 7)**	94 ± 9 **(-6 ± 9)**	94 ± 12 **(-6 ± 12)**	97 ± 10 **(-4 ± 10)**

Statistical comparison = saline : granisetron

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Table 5.10 **Buspirone 200 μg kg^{-1} i.c.** **(WAY-100635 100 μg kg^{-1} i.v. pretreated, 20 minutes previously) n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	62 ± 6	55 ± 5 **(-7 ± 4)**	45 ± 2 **(-18 ± 4)**	46 ± 3 **(-16 ± 5)**	49 ± 3 **(-13 ± 4)**
R-R interval (ms)	256 ± 6	262 ± 4 **(6 ± 3*)**	263 ± 4 **(7 ± 3)**	260 ± 5 **(4 ± 5)**	260 ± 5 **(4 ± 4)**
Phrenic burst rate(bursts min^{-1})	55 ± 2	52 ± 3** **(-3 ± 3*)**	56 ± 3** **(1 ± 2**)**	57 ± 4** **(2 ± 2**)**	58 ± 4** **(3 ± 2**)**
RNA (%)	100	113 ± 4* **(13 ± 4*)**	86 ± 7 **(-14 ± 7)**	81 ± 10 **(-19 ± 10)**	86 ± 9* **(-14 ± 9)**

Statistical comparison = buspirone : buspirone + WAY-100635

Table 5.11 **Sumatriptan 50 μg kg^{-1} i.c.** **(WAY-100635 100 μg kg^{-1} i.v. pretreated, 20 minutes previously) n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	49 ± 6	49 ± 7 **(1 ± 3)**	54 ± 5 **(5 ± 2*)**	54 ± 6 **(6 ± 2*)**	60 ± 8 **(11 ± 3*)**
R-R interval (ms)	250 ± 8	248 ± 9 **(-2 ± 1)**	249 ± 10 **(-1 ± 4)**	249 ± 10 **(-1 ± 5)**	250 ± 9 **(0 ± 5)**
Phrenic burst rate(bursts min^{-1})	52 ± 3	54 ± 4 **(2 ± 3)**	73 ± 5* **(22 ± 6)**	91 ± 16** **(40 ± 17**)**	97 ± 13** **(46 ± 14**)**
RNA (%)	100	111 ± 4* **(11 ± 4*)**	140 ± 11** **(40 ± 11**)**	141 ± 9** **(41 ± 9**)**	152 ± 12** **(52 ± 12**)**

Statistical comparison = saline : sumatriptan + WAY-100635

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Table 5.12 **(+)8-OH-DPAT 25 μg kg^{-1} i.c.** (**GR-127935 100 μg kg^{-1} i.v.** pretreated 20 minutes previously) n=4.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	46±2	43±1 **(-3±1)**	52±4 **(6±3)**	49±4 **(4±3)**	46±2 **(0±1)**
R-R interval (ms)	265±6	280±7 **(15±5)**	286±7 **(21±7)**	285±6 **(20±8)**	291±7 **(26±9)**
Phrenic burst rate(bursts min^{-1})	66±13	162±27 **(97±15)**	185±25 **(119±15)**	178±15 **(113±4)**	165±17 **(100±8)**
RNA (%)	100	77±15 **(-23±15)**	174±46** **(74±46**)**	166±42****(91±67**)**	152±26***(52±25)**

Statistical comparison = 8-OH-DPAT : 8-OH-DPAT + GR-127935

Table 5.13 **(+)8-OH-DPAT 25 μg kg^{-1} i.c.** (**WAY-100635 100 μg kg^{-1} i.v.** pretreated 20 minutes previously) n=4.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	69±4	59±5* **(-10±4)**	59±4 **(-10±3)**	56±3 **(-13±3*)**	56±3 **(-14±3)**
R-R interval (ms)	269±11	269±11 **(0±1)**	271±11 **(0±1*)**	278±10 **(9±2)**	278±11 **(9±5)**
Phrenic burst rate(bursts min^{-1})	59±3	73±8 **(14±7)**	97±12* **(38±11*)**	108±14 **(49±13*)**	140±14 **(79±16)**
RNA (%)	100	99±6** **(-2±6**)**	93±7* **(-8±7*)**	90±6 **(-10±6)**	97±9 **(-4±9)**

Statistical comparison = 8-OH-DPAT : 8-OH-DPAT + WAY-100635

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Table 5.14 **Acidified saline 20 μl i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	48±5	48±5 **(-1±1)**	45±4 **(-4±2)**	43±3 **(-5±2)**	40±2 **(-9±5)**
R-R interval (ms)	273±5	270±4 **(-3±1)**	270±4 **(-3±3)**	270±2 **(-3±4)**	271±2 **(-2±3)**
Phrenic burst rate(bursts min^{-1})	38±3	44±2 **(6±4)**	47±2 **(9±4)**	47±3 **(9±5)**	47±3 **(9±4)**
RNA (%)	100	100±6 **(1±5)**	101±6 **(4±6)**	104±4 **(5±6)**	88±11 **(-9±13)**

Table 5.15 **Sulpiride 200 μg kg^{-1} i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	39±2	46±1 **(7±3)**	53±3 **(14±5**)**	53±3 **(14±5**)**	53±3** **(14±5**)**
R-R interval (ms)	260±6	254±7* **(-6±3)**	248±7** **(-13±3*)**	245±5** **(-15±2)**	244±4** **(-19±6*)**
Phrenic burst rate(bursts min^{-1})	46±5	59±6* **(13±5)**	59±7 **(13±2)**	61±7* **(15±3)**	57±7 **(11±3)**
RNA (%)	100	142±35 **(42±35)**	149±28 **(49±28)**	134±18 **(34±18)**	158±31* **(58±31*)**

Statistical comparison = acidified saline : sulpiride

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Table 5.16 **(-)Pindolol 100 μg kg-1 i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	41±2	35±1** (-6±4)	29±2** (-12±3)	28±2** (-13±3)	30±3* (-11±4)
R-R interval (ms)	265±3	277±4 (12±4*)	273±4 (8±5)	270±8 (5±9)	272±7 (7±8)
Phrenic burst rate(bursts min^{-1})	54±8	46±4 (-8±5)	40±9 (-13±9*)	38±8 (-16±8**)	29±8* (-26±9**)
RNA (%)	100	84±18 (-16±18)	67±17* (-33±17*)	55±17** (-45±17**)	55±14 (-45±18)

Statistical comparison = acidified saline : pindolol

Table 5.17 **Distilled water 20 μl i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	65±3	63±3 (-1±2)	63±3 (-2±3)	59±2 (-6±3)	58±2 (-6±3)
R-R interval (ms)	264±6	259±7 (-5±2)	256±8 (-8±3)	254±7 (-10±4)	253±7 (-11±2)
Phrenic burst rate(bursts min^{-1})	67±3	68±6 (2±5)	68±6 (2±6)	68±6 (1±6)	69±6 (2±6)
RNA (%)	100	99±6 (-1±6)	103±9 (3±9)	97±11 (-3±11)	95±12 (-5±12)

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Table 5.18 **Mesulergine 200 μg kg^{-1} i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	58±4	60±6 **(3±3)**	57±7 **(-1±5)**	54±6 **(-3±3)**	53±5 **(-4±3)**
R-R interval (ms)	283±43	276±44 **(-6±5)**	275±43 **(-8±5)**	273±36 **(-10±7)**	270±31 **(-13±13)**
Phrenic burst rate(bursts min^{-1})	47±8	50±9 **(3±3)**	47±8 **(1±3)**	55±11 **(8±6)**	56±9 **(9±6)**
RNA (%)	100	133±23* **(30±24)**	113±6 **(13±6)**	94±13 **(-6±13)**	94±10 **(-6±10)**

Statistical comparison = distilled water : mesulergine

Table 5.19 **GR-127935 20 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	65±5	60±4 **(-4±1)**	59±4 **(-6±2)**	58±4 **(-6±2)**	59±3 **(-6±3)**
R-R interval (ms)	252±7	253±6 **(1±1)**	253±6 **(1±1)**	254±6 **(2±3*)**	255±5 **(3±3**)**
Phrenic burst rate(bursts min^{-1})	71±7	68±3 **(-3±5)**	68±3 **(-3±5)**	68±3 **(-3±6)**	68±3 **(-2±8)**
RNA (%)	100	98±4 **(-2±4)**	98±7 **(-2±7)**	97±10 **(-3±10)**	96±12 **(-4±12)**

Statistical comparison = distilled water : GR-127935

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Table 5.20 **GR-127935 100 μg kg^{-1} i.v. n=4.**

Time after drug (min)	-5	5	15
MAP (mmHg)	55±6	53±7 **(-2±1)**	50±4 **(-5±3)**
R-R interval (ms)	273±11	273±10 **(0±4)**	271±10 **(-1±5)**
Phrenic burst rate(bursts min^{-1})	66±14	66±14 **(1±1)**	68±12 **(2±3)**
RNA (%)	100	96±2 **(-4±2)**	90±2 **(-10±2)**

Statistical comparison = distilled water : GR-127935

Table 5.21 **Atenolol 1 mg kg^{-1} i.v. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	76±4	71±4 **(-5±1)**	70±7 **(-5±4)**	67±6 **(-8±3*)**	67±5 **(-8±2*)**
R-R interval (ms)	216±4	283±12** **(67±10**)**	288±15** **(72±12**)**	286±15** **(70±12**)**	280±14** **(64±11**)**
Phrenic burst rate(bursts min^{-1})	68±15	61±9 **(-7±7)**	60±8 **(-8±7)**	58±7 **(-10±11)**	57±6 **(-11±10)**
RNA (%)	100	101±9 **(1±9)**	110±13 **(11±13)**	102±9 **(2±9)**	99±8 **(0±8)**

Statistical comparison = 5 min before atenolol : atenolol at indicated time

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Table 5.22 Size of reflex before drug.

Drug	Dose and route	n	Increase MAP (mmHg)	Increase R-R interval (ms)	Apnoea duration (s)
Saline (Vehicle)	20 μl i.c.	5	22±4	128±41	25±5
Buspirone	200 μg kg^{-1} i.c.	5	29±1	135±26	22±5
(+)8-OH-DPAT	25 μg kg^{-1} i.c.	5	23±3	141±22	21±3
WAY-100635	100 μg kg^{-1} i.c.	5	32±2	158±43	22±2
WAY-100635	100 μg kg^{-1} i.v.	5	29±3	77±19	22±3
nn-DP-5-CT	50 μg kg^{-1} i.c.	5	31±1	142±27	26±6
Granisetron	20 μg kg^{-1} i.c.	5	28±2	198±53	19±2
Granisetron	20 μg kg^{-1} i.v.	5	30±4	79±27	21±6
Buspirone (after WAY-100635)	200 μg kg^{-1} i.c. (100 μg kg^{-1} i.v.)	5	24±3	62±12	19±3
Sumatriptan (after WAY-100635)	50 μg kg^{-1} i.c. (100 μg kg^{-1} i.v.)	5	28±2	131±33	19±4
(+)8-OH-DPAT (after GR-127935)	25 μg kg^{-1} i.c. (100 μg kg^{-1} i.v.)	4	26±3	88±17	15±4

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Drug	Dose and route	n	Increase MAP (mmHg)	Increase R-R interval (ms)	Apnoea duration (s)
(+)8-OH-DPAT (after WAY-100635)	25 μg kg^{-1} i.c. (100 μg kg^{-1} i.v.)	4	19±5	114±48	19±2
Acidified saline (Vehicle)	20 μl i.c.	5	25±1	167±51	29±5
Sulpiride	200 μg kg^{-1} i.c.	4	23±4	70±9	19±3
(-)Pindolol	100 μg kg^{-1}	5	27±3	218±60	29±6
Distilled water (Vehicle)	20 μl i.c.	4	32±3	90±53	18±6
Mesulergine	200 μg kg^{-1} i.c.	4	24±2	88±33	18±9
GR-127935	20 μg kg^{-1} i.c.	5	23±2	56±15	10±2
GR-127935	100 μg kg^{-1} i.v.	4	29±3	86±32	20±1
Atenolol	1 mg kg i.v.	5	22±2	107±35	11±3

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Size of reflex after drug.

Table 5.23 **Saline 20 µl i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	22 ± 4	22 ± 5 (-1±1)	22 ± 5 (0±1)	23 ± 4 (0±1)	21 ± 5 (-1±2)
Increase R-R interval (ms)	128 ± 41	124 ± 45 (-4±9)	119 ± 46 (-7±11)	129 ± 38 (2±9)	119 ± 36 (-6±10)
3s R-R increase (ms)	7 ± 2	7 ± 2 (0 ± 1)	11 ± 6 (4 ± 4)	10 ± 5 (2 ± 4)	10 ± 6 (3 ± 5)
Apnoea duration (s)	25 ± 5	26 ± 5 (1±2)	24 ± 4 (-1±2)	24 ± 5 (0±1)	23 ± 5 (-2±2)
RNA (%)	100	97 ± 8 (-5±7)	102 ± 4 (-6±6)	103 ± 4 (-6±8)	102 ± 5 (-7±8)

Table 5.24 **Buspirone 200 µg kg-1 i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	29 ± 1	24 ± 1 (-5±1*)	29 ± 3 (-0±3)	22 ± 1 (-6±0)	25 ± 0 (-3±1)
Increase R-R interval (ms)	135 ± 26	324 ± 86*(162±82*)	283 ± 83 (175±63**)	285 ± 73 (183±53**)	318 ± 76*(201±83**)
3s R-R increase (ms)	15 ± 4	71 ± 17**(55±19**)	72 ± 18**(57±18**)	54 ± 21**(39±18*)	39 ± 13(23±11)
Apnoea duration (s)	22 ± 5	15 ± 4 (-7±5)	17 ± 5 (-4±5)	16 ± 3 (-6±4)	16 ± 3 (-5±4)
RNA (%)	100	121 ± 15 (21±15)	98 ± 8 (-2±8)	101 ± 8 (1±8)	118 ± 22 (18±22)

Statistical comparison = saline : buspirone

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Table 5.25 **(+)8-OH-DPAT 25 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	23 ± 3	16 ± 4 **(-6±3)**	13 ± 2 **(-10±2*)**	14 ± 3 **(-8±3)**	9 ± 7 **(-13±5**)**
Increase R-R interval (ms)	141 ± 22	43 ± 11 **(-98±23**)**	38 ± 7 **(-103±21**)**	37 ± 7* **(-104±22**)**	49 ± 13 **(-93±24**)**
3s R-R increase (ms)	9 ± 3	16 ± 11 **(7±11)**	10 ± 5 **(1±5)**	11 ± 3 **(2±5)**	14 ± 5 **(5±7)**
Apnoea duration (s)	21 ± 3	10 ± 2** **(-11±3*)**	8 ± 2** **(-13±2**)**	7 ± 2** **(-14±3**)**	5 ± 1** **(-16±4**)**
RNA (%)	100	52 ± 11****(-51±10**)**	59 ± 9** **(-41±9**)**	61 ± 7** **(-39±7**)**	76 ± 13* **(-24±13)**

Statistical comparison = saline : 8-OH-DPAT

Table 5.26 **WAY-100635 100 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	32 ± 2	22 ± 7 **(-9±5*)**	26 ± 4 **(-5±3)**	28 ± 3 **(-3±1)**	29 ± 2 **(-3±1)**
Increase R-R interval (ms)	158 ± 43	91 ± 44 **(-68±27**)**	105 ± 43 **(-57±24*)**	130 ± 31 **(-28±14)**	108 ± 14 **(-51±30)**
Apnoea duration (s)	22 ± 2	25 ± 6 **(3±6)**	19 ± 4 **(-3±3)**	19 ± 2 **(-2±2)**	20 ± 2 **(-2±1)**
RNA (%)	100	100 ± 16 **(0±16)**	92 ± 9 **(-8±9)**	99 ± 2 **(-1±2)**	100 ± 6 **(0±6)**

Statistical comparison = saline : WAY-100635

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Table 5.27 **WAY-100635 100 μg kg-1 i.v. n=5.**

Time after drug (min)	-5	5	15
Increase MAP (mmHg)	29 ± 3	24 ± 2 **(-5±1)**	28 ± 3 **(-1±1)**
Increase R-R interval (ms)	77 ± 19	72 ± 18 **(-5±7)**	61 ± 13 **(-16±11)**
Apnoea duration (s)	22 ± 3	20 ± 4 **(-1±2)**	20 ± 3 **(-2±2)**
RNA (%)	100	97 ± 1 **(-3±1)**	96 ± 3 **(-4±3)**

Statistical comparison = saline : WAY-100635

Table 5.28 **nn-DP-5-CT 50 μg kg-1 i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	31 ± 1	25 ± 3 **(-8±2*)**	19 ± 4 **(-16±3**)**	11 ± 5* **(-22±3**)**	8 ± 5* **(-26±4**)**
Increase R-R interval (ms)	142 ± 27	97 ± 20 **(-45±24)**	84 ± 25 **(-58±32)**	94 ± 27 **(-48±30)**	93 ± 36 **(-49±41)**
Apnoea duration (s)	26 ± 6	17 ± 5* **(-8±4)**	16 ± 5* **(-10±5)**	17 ± 4* **(-8±6)**	13 ± 4* **(-12±6)**
RNA (%)	100	99 ± 5 **(-1±5)**	72 ± 16** **(-28±16)**	67 ± 11** **(-33±11*)**	64 ± 11** **(-36±11*)**

Statistical comparison = saline : DP-5-CT

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Table 5.29 **Granisetron 20 μg kg-1 i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	28 ± 2	30 ± 3 **(2±3)**	24 ± 2 **(-3±1)**	26 ± 2 **(-2±2)**	28 ± 2 **(0±1)**
Increase R-R interval (ms)	198 ± 53	110 ± 46 **(-88±51)**	77 ± 32 **(-121±55*)**	86 ± 29 **(-112±53*)**	147 ± 56 **(-51±77)**
Apnoea duration (s)	19 ± 2	16 ± 3 **(-3±2)**	11 ± 2* **(-7±3*)**	13 ± 2* **(-6±2)**	13 ± 3 **(-6±2)**
RNA (%)	100	112 ± 9 **(12±9)**	109 ± 13 **(9±13)**	115 ± 15 **(15±15)**	117 ± 16 **(17±16)**

Statistical comparison = saline : granisetron

Table 5.30 **Granisetron 20 μg kg-1 i.v. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	30 ± 4	30 ± 4 **(0±1)**	28 ± 5 **(-2±3)**	27 ± 5 **(-3±3)**	28 ± 4 **(-2±2)**
Increase R-R interval (ms)	79 ± 27	88 ± 40 **(9±19)**	93 ± 40 **(14±18)**	80 ± 38 **(1±19)**	84 ± 34 **(5±13)**
Apnoea duration (s)	21 ± 6	20 ± 7 **(-1±2)**	20 ± 9 **(-1±3)**	19 ± 10 **(-1±4)**	19 ± 9 **(-2±3)**
RNA (%)	100	94 ± 4 **(-6±4)**	100 ± 7 **(0±7)**	94 ± 8 **(-6±8)**	98 ± 5 **(-2±5)**

Statistical comparison = saline : granisetron

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Table 5.31 **Buspirone 200 μg kg-1 i.c.** (**WAY-100635 100 μg kg-1 i.v.** pretreated 20 mins previously) n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	24 ± 3	28 ± 2 **(4±2**)**	26 ± 3 **(1±2)**	28 ± 3* **(4±2**)**	27 ± 3 **(2±2*)**
Increase R-R interval (ms)	62 ± 12	83 ± 25****(21±19**)**	78 ± 27****(15±20*)**	82 ± 20* **(20±11*)**	89 ± 21****(27±12*)**
3s R-R increase (ms)	5 ± 3	5 ± 1****(0 ± 1**)**	5 ± 1****(-1 ± 2**)**	5 ± 2****(0 ± 4*)**	4 ± 0***(-1 ± 2)**
Apnoea duration (s)	19 ± 3	18 ± 2 **(-1±3)**	16 ± 3 **(-4±2)**	17 ± 2 **(-3±2)**	17 ± 2 **(-3±2)**
RNA (%)	100	109 ± 3 **(9±3)**	103 ± 7 **(3±7)**	97 ± 8 **(-3±8)**	97 ± 10 **(-3±10)**

Statistical comparison = buspirone : buspirone + WAY-100635

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Table 5.32 **Sumatriptan 50 μg kg-1 i.c.** (**WAY-100635 100 μg kg-1 i.v.** pretreated 20 mins previously) n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	28 ± 3	23 ± 3 **(-5±1)**	16 ± 2 **(-12±3**)**	22 ± 3 **(-6±2)**	19 ± 2 **(-8±2*)**
Increase R-R interval (ms)	131 ± 33	82 ± 28 **(-48±16)**	72 ± 23 **(-59±14*)**	55 ± 11 **(-76±24**)**	43 ± 10 **(-88±35**)**
Apnoea duration (s)	19 ± 4	13 ± 5 **(-6±3)**	13 ± 5 **(-5±3)**	11 ± 4* **(-8±2)**	8 ± 4* **(-11±5*)**
RNA (%)	100	98 ± 4 **(-2±4)**	106 ± 7 **(6±7)**	109 ± 10 **(9±10)**	112 ± 12 **(12±12)**

Statistical comparison = saline : sumatriptan + WAY-100635

Table 5.33 **(+)8-OH-DPAT 25 μg kg-1 i.c.** (**GR-127935 100 μg kg-1 i.v.** pretreated 20 mins previously) n=4.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	26 ± 3	13 ± 4 **(-13±4)**	14 ± 4 **(-12±5)**	14 ± 3 **(-12±4)**	16 ± 4 **(-10±5)**
Increase R-R interval (ms)	88 ± 17	91 ± 29* **(3±21**)**	82 ± 25 **(-6±23**)**	48 ± 20 **(-40±30*)**	63 ± 16 **(-25±8*)**
3s R-R increase (ms)	8 ± 2	10 ± 2 **(3±2)**	16 ± 7 **(9±6)**	19 ± 7 **(12±6)**	20 ± 11 **(12±11)**
Apnoea duration (s)	15 ± 4	8 ± 0 **(-7±4)**	6 ± 0 **(-9±4)**	6 ± 1 **(-9±5)**	3 ± 1 **(-11±3)**
RNA (%)	100	75 ± 9* **(-25±9*)**	89 ± 4* **(-11±4*)**	95 ± 7** **(-5±7**)**	97 ± 9 **(-3±9)**

Statistical comparison = 8-OH-DPAT : 8-OH-DPAT + GR-127935

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Table 5.34 **(+)8-OH-DPAT 25 μg kg-1 i.c.** (**WAY-100635 100 μg kg-1 i.v.** pretreated 20 min previously) n=4.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	19±5	14±5 **(-5±4)**	13±5 **(-6±3)**	12±5 **(-7±3)**	14±5 **(-5±3)**
Increase R-R interval (ms)	114±48	46±32 **(-68±30)**	26±13 **(-87±40)**	34±15 **(-80±35)**	30±16 **(-84±37)**
3s R-R increase (ms)	15±9	5±4 **(-10±11)**	10±7 **(-5±12)**	7±5 **(-8±11)**	11±6 **(-4±13)**
Apnoea duration (s)	19±2	15±3 **(-4±1)**	14±3 **(-5±1*)**	13±2 **(-6±1*)**	14±2 **(-5±1**)**
RNA (%)	100	98±14** **(-2±14**)**	100±3** **(0±3**)**	91±4* **(-9±4**)**	83±5 **(-17±5)**

Statistical comparison = 8-OH-DPAT : 8-OH-DPAT + WAY-100635

Table 5.35 **Acidified saline 20 μl i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	25 ± 1	23 ± 2 **(-2±1)**	23 ± 3 **(-2±1)**	22 ± 2 **(-3±1)**	22 ± 3 **(-6±7)**
Increase R-R interval (ms)	167 ± 51	170 ± 50 (14±27)	169 ± 56 **(2±15)**	159 ± 52 **(-8±11)**	107 ± 39 **(-60±38)**
Apnoea duration (s)	29 ± 5	29 ± 5 **(3±4)**	26 ± 6 **(1±3)**	24 ± 5 **(-1±4)**	18 ± 3 **(-7±7)**
RNA (%)	100	101 ± 5 **(6±3)**	108 ± 4 **(6±4)**	101 ± 6 **(2±6)**	93 ± 6 **(-6±7)**

Table 5.36 **Sulpiride 200 μg kg^{-1} i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	20 ± 3	24 ± 3 **(4±2)**	25 ± 4 **(4±3)**	25 ± 5 **(4±5)**	27 ± 5 **(7±5*)**
Increase R-R interval (ms)	70 ± 9	77 ± 6 **(7±12)**	82 ± 8 **(12±17)**	54 ± 8 **(-16±14)**	65 ± 6 **(-5±15)**
Apnoea duration (s)	19 ± 3	13 ± 2* **(-6±2)**	7 ± 3** **(-12±2)**	1 ± 1** **(-18±4*)**	2 ± 2** **(-17±4)**
RNA (%)	100	100 ± 15 **(-1±15)**	97 ± 12 **(-4±12)**	97 ± 15 **(-4±15)**	110 ± 16 **(10±16)**

Statistical comparison = acidified saline : sulpiride

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Table 5.37 **(-)Pindolol 100 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	27 ± 3	21 ± 2 **(-6±4)**	11 ± 3** **(-16±5**)**	7 ± 3** **(-20±6**)**	4 ± 3** **(-23±5**)**
Increase R-R interval (ms)	218 ± 60	109 ± 41 **(-109±27)**	11 ± 5* **(-207±60**)**	8 ± 2* **(-210±61**)**	11 ± 4 **(-207±60**)**
Apnoea duration (s)	29 ± 6	33 ± 12 **(4±7)**	35 ± 8 **(5±9)**	54 ± 12** **(24±7**)**	42 ± 7* **(13±8*)**
RNA (%)	100	85 ± 15 **(-15±14)**	61 ± 10****(-41±12**)**	41 ± 12****(-59±12**)**	35 ± 12****(-61±13**)**

Statistical comparison = acidified saline : pindolol

Table 5.38 **Distilled water 20 μl i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	32 ± 3	25 ± 2 **(-7±4)**	28 ± 2 **(-7±2)**	29 ± 1 **(-3±3)**	19 ± 6 **(-13±5)**
Increase R-R interval (ms)	90 ± 53	79 ± 60 **(-11±11)**	73 ± 37 **(-17±16)**	50 ± 17 **(-40±37)**	66 ± 43 **(-24±10)**
Apnoea duration (s)	18 ± 6	12 ± 6 **(-5±1)**	13 ± 4 **(-5±3)**	11 ± 2 **(-7±4)**	7 ± 4 **(-11±3)**
RNA (%)	100	97 ± 6 **(-4±6)**	98 ± 8 **(-2±8)**	96 ± 6 **(-4±6)**	90 ± 6 **(-10±6)**

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Table 5.39 **Mesulergine 200 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	24 ± 2	26 ± 5 **(2±3)**	36 ± 7 **(13±6)**	22 ± 5 **(-2±3)**	25 ± 5 **(1±3)**
Increase R-R interval (ms)	88 ± 33	50 ± 26 **(-38±33)**	42 ± 20 **(-46±33)**	48 ± 13 **(-41±32)**	51 ± 16 **(-38±31)**
Apnoea duration (s)	18 ± 9	15 ± 9 **(-3±1)**	17 ± 4 **(-1±5)**	8 ± 5 **(-11±5)**	12 ± 6 **(-6±6)**
RNA (%)	100	111 ± 5 **(11±5)**	128 ± 13* **(28±13)**	97 ± 13 **(-2±13)**	90 ± 16 **(-10±16)**

Statistical comparison = distilled water : mesulergine

Table 5.40 **GR-127935 20 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	23 ± 2	27 ± 4 **(4±3*)**	26 ± 3 **(3±2)**	25 ± 4 **(2±4)**	24 ± 5 **(1±4**)**
Increase R-R interval (ms)	56 ± 15	88 ± 32 **(31±19)**	78 ± 36 **(22±26)**	101 ± 50 **(47±38*)**	84 ± 36 **(28±23)**
Apnoea duration (s)	10 ± 2	11 ± 3 **(0±2)**	9 ± 4 **(-1±3)**	12 ± 7 **(2±5*)**	10 ± 4 **(-1±3*)**
RNA (%)	100	99 ± 6 **(-1±6)**	101 ± 12 **(1±12)**	103 ± 15 **(3±15)**	94 ± 15 **(-6±15)**

Statistical comparison = distilled water : GR-127935

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Table 5.41 **GR-127935 100 μg kg-1 i.v. n=4.**

Time after drug (min)	-5	5	15
Increase MAP (mmHg)	29 ± 3	26 ± 4 **(-3±2)**	26 ± 3 **(-3±1)**
Increase R-R interval (ms)	85 ± 32	82 ± 19 **(-4±14)**	88 ± 17 **(2±37)**
Apnoea duration (s)	20 ± 1	18 ± 3 **(-2±2)**	15 ± 4 **(-5±3)**
RNA (%)	100	93 ± 3 **(-7±3)**	87 ± 3* **(-13±3)**

Statistical comparison = distilled water : GR-127935

Table 5.42 **Atenolol 1 mg kg-1 i.v. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	22 ± 2	24 ± 3 **(2±2)**	27 ± 3 **(6±2*)**	28 ± 2 **(6±3*)**	26 ± 1 **(4±1)**
Increase R-R interval (ms)	107 ± 35	71 ± 38 **(-36±9)**	76 ± 21 **(-31±17)**	86 ± 13 **(-21±36)**	108 ± 28 **(0±16)**
Apnoea duration (s)	11 ± 3	13 ± 3 **(1±0)**	17 ± 5 **(6±2*)**	16 ± 5 **(5±3*)**	17 ± 3 **(6±1*)**
RNA (%)	100	109 ± 8 **(9±8)**	114 ± 6* **(14±6*)**	107 ± 7 **(7±7)**	101 ± 3 **(1±3)**

Statistical comparison = 5 min before atenolol : atenolol at time indicated

Data obtained from anaesthetized and ventilated rabbits, stimulated with smoke.

Table 5.43 Resting baseline values, before drug.

Drug	Dose and route	n	Baseline MAP (mmHg)	Baseline R-R interval (ms)	Baseline respiratory rate (breaths min^{-1})
Saline	20 µl i.c.	5	55 ± 3	265 ± 5	53 ± 3
Buspirone	200 µg kg^{-1} i.c.	4	60 ± 5	258 ± 5	59 ± 4

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Resting baseline values following drug.

Table 5.44 **Saline 20 μl i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	55 ± 3	56 ± 2 **(1±1)**	59 ± 2 **(4±2)**	59 ± 2 **(5±3)**	57 ± 2 **(2±4)**
R-R interval (ms)	265 ± 5	267 ± 5 **(2±2)**	267 ± 8 **(2±5)**	266 ± 8 **(1±5)**	268 ± 7 **(3±2)**
Phrenic burst rate(bursts min^{-1})	53 ± 3	50 ± 3 **(-3±2)**	54 ± 3 **(1±2)**	53 ± 3 **(0±2)**	53 ± 3 **(-1±2)**
RNA (%)	100	116 ± 25 **(16±25)**	82 ± 20 **(-19±20)**	101 ± 5 **(2±5)**	125 ± 18 **(25±18)**

Table 5.45 **Buspirone 200 μg kg^{-1} i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	60 ± 5	56 ± 5 **(-4±1)**	52 ± 3 **(-8±2**)**	52 ± 3 **(-8±2**)**	53 ± 4 **(-7±2*)**
R-R interval (ms)	258 ± 5	294 ± 18* **(36±18**)**	289 ± 7 **(31±7*)**	279 ± 8 **(21±10)**	276 ± 7 **(19±8)**
Phrenic burst rate(bursts min^{-1})	59 ± 5	63 ± 4* **(4±1)**	63 ± 1 **(5±5)**	60 ± 5 **(2±1)**	61 ± 5 **(3±3)**
RNA (%)	100	114 ± 28 **(14±28)**	107 ± 36 **(7±36)**	94 ± 19 **(-6±19)**	107 ± 13 **(7±13)**

Statistical comparison = saline : buspirone

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Table 5.46 Size of reflex before drug.

Drug	Dose and route	n	Increase MAP (mmHg)	Increase R-R interval (ms)	Apnoea duration (s)
Saline	20 µl i.c.	5	19 ± 5	41 ± 18	29 ± 5
Buspirone	200 µg kg^{-1} i.c.	4	20 ± 3	51 ± 19	24 ± 7

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Size of reflex following drug.

Table 5.47 **Saline 20 μl i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	19 ± 5	19 ± 3 **(0±1)**	17 ± 2 **(-2±3)**	17 ± 4 **(-2±3)**	19 ± 4 **(0±1)**
Increase R-R interval (ms)	41 ± 18	40 ± 15 **(-1±3)**	33 ± 8 **(-8±10)**	41 ± 12 **(-1±6)**	37 ± 8 **(-5±10)**
Apnoea duration (s)	29 ± 5	23 ± 3 **(-5±7)**	26 ± 4 **(-3±7)**	23 ± 5 **(-5±6)**	25 ± 6 **(-3±7)**
RNA (%)	100	118 ± 6 **(18±6)**	109 ± 18 **(9±18)**	116 ± 17 **(16±17)**	118 ± 19 **(18±19)**

Table 5.48 **Buspirone 200 μg kg^{-1} i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	20 ± 3	14 ± 3 **(-7±4)**	16 ± 2 **(-4±2)**	18 ± 2 **(-2±3)**	20 ± 5 **(0±4)**
Increase R-R interval (ms)	51 ± 19	122 ± 58* **(71±56*)**	80 ± 28 **(29±29)**	70 ± 13 **(19±19)**	66 ± 22 **(16±26)**
Apnoea duration (s)	24 ± 7	22 ± 10 **(-2±6)**	13 ± 7 **(-11±7)**	7 ± 4* **(-18±6)**	7 ± 4* **(-17±3)**
RNA (%)	100	80 ± 6* **(-21±6*)**	64 ± 6* **(-36±6*)**	78 ± 13* **(-23±13)**	86 ± 11 **(-14±11)**

Statistical comparison = saline : buspirone

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Data obtained from anaesthetized, hyperoxic rabbits, stimulated with smoke.

Table 5.49 Baseline values before drug.

Drug	Dose and route	n	Baseline MAP (mmHg)	Baseline R-R interval (ms)	Baseline respiratory rate (breaths min^{-1})
Saline	20 μl i.c.	4	58 ± 3	266 ± 5	69 ± 5
Buspirone	200 μg kg^{-1} i.c.	4	58 ± 6	260 ± 17	60 ± 8

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Baseline values after drug.

Table 5.50 **Saline 20 μl i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	58 ± 3	55 ± 3 **(-2±2)**	53 ± 3 **(-4±2)**	51 ± 4 **(-5±3)**	51 ± 5 **(-4±3)**
R-R interval (ms)	266 ± 5	263 ± 6 **(-3±2)**	261 ± 7 **(-5±5)**	256 ± 7 **(-10±7)**	257 ± 7 **(-9±7)**
Phrenic burst rate(bursts min^{-1})	69 ± 5	62 ± 2 **(-7±6)**	66 ± 6 **(-3±2)**	63 ± 4 **(-6±3)**	66 ± 5 **(-3±3)**
RNA (%)	100	105 ± 7 **(30±30)**	104 ± 15 **(4±15)**	89 ± 19 **(-8±18)**	92 ± 20 **(-8±20)**

Table 5.51 **Buspirone 200 μg kg^{-1} i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	58 ± 6	52 ± 6 **(-12±4*)**	53 ± 5 **(-10±1)**	56 ± 6 **(-9±3)**	57 ± 6 **(-9±2)**
R-R interval (ms)	260 ± 17	314 ± 23* **(54±16**)**	295 ± 29 **(35±13**)**	269 ± 21 **(9±4)**	258 ± 18 **(-3±3)**
Phrenic burst rate(bursts min^{-1})	60 ± 8	77 ± 8 **(17±13*)**	76 ± 5 **(16±8)**	81 ± 7 **(21±10*)**	82 ± 11 **(22±8*)**
RNA (%)	100	88 ± 34 **(-12±34)**	67 ± 21 **(-33±21)**	58 ± 19 **(-42±19)**	12 ± 56 **(-44±12)**

Statistical comparison = saline : buspirone

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Table 5.52 Size of reflex before drug.

Drug	Dose and route	n	Increase in MAP (mmHg)	Increase in R-R interval (ms)	Apnoea duration (s)
Saline	20 μl i.c.	4	22 ± 4	72 ± 20	16 ± 4
Buspirone	200 μg kg^{-1} i.c.	4	19 ± 2	70 ± 8	18 ± 4

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Size of reflex after drug.

Table 5.53 **Saline 20 μl i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	22 ± 4	25 ± 2 **(3±2)**	27 ± 1 **(5±4)**	23 ± 2 **(1±4)**	23 ± 3 **(1±2)**
Increase R-R interval (ms)	72 ± 20	73 ± 18 **(0±4)**	73 ± 11 **(0±9)**	61 ± 19 **(-11±10)**	68 ± 21 **(-5±3)**
Apnoea duration (s)	16 ± 4	16 ± 1 **(1±3)**	21 ± 5 **(5±6)**	14 ± 6 **(-2±5)**	16 ± 5 **(0±2)**
RNA (%)	100	113 ± 37 **(13±27)**	153 ± 73 **(53±73)**	115 ± 49 **(15±49)**	122 ± 45 **(22±45)**

Table 5.54 **Buspirone 200 μg kg-1 i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	19 ± 2	17 ± 3* **(-2±1)**	19 ± 2* **(0±2)**	17 ± 4 **(-2±2)**	20 ± 2 **(1±1)**
Increase R-R interval (ms)	70 ± 8	126 ± 38 **(49±30)**	173±31**(**103±24**)**	156 ± 33* **(86±26**)**	155 ± 30* **(86±23**)**
Apnoea duration (s)	18 ± 4	14 ± 1 **(-4±4)**	22 ± 7 **(12±15)**	19 ± 4 **(1±6)**	21 ± 3 **(4±3)**
RNA (%)	100	146 ± 80 **(46±80)**	203 ± 102 **(103±102)**	111 ± 43 **(11±43)**	106 ± 35 **(6±35)**

Statistical comparison = saline : buspirone

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Data obtained from anaesthetized rabbits, stimulated with phenyl biguanide into the right atrium.

Table 5.55 Resting baseline values.

Drug	Dose and route	n	Baseline MAP (mmHg)	Baseline R-R interval (ms)
Saline	20 µl i.c.	5	59 ± 5	268 ± 7
Buspirone	200 µg kg^{-1}	5	52 ± 2	253 ± 5

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Resting baseline values after drug.

Table 5.56 **Saline 20 μl i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	59 ± 5	57 ± 5 **(-2±2)**	56 ± 6 **(-3±3)**	55 ± 6 **(-4±4)**	57 ± 7 **(-2±5)**
R-R interval (ms)	268	269 ± 7 **(1±1)**	273 ± 8 **(5±2)**	277 ± 8 **(9±3)**	276 ± 6 **(8±3)**

Table 5.57 **Buspirone 200 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	52 ± 2	51 ± 6 **(-1±5)**	45 ± 2 **(-7±3)**	47 ± 2 **(-6±3)**	53 ± 4 **(0±6)**
R-R interval (ms)	253	262 ± 8 **(17±4*)**	265 ± 6 **(20±4*)**	262 ± 7 **(17±5)**	257 ± 9 **(12±7)**

Statistical comparison = saline : buspirone

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Table 5.58 Size of reflex before drug.

Drug	Dose and route	n	Decrease in MAP (mmHg)	Increase in R-R interval (ms)
Saline	20 µl i.c.	5	-23 ± 5	37 ± 7
Buspirone	200 µg kg^{-1} i.c.	5	-17 ± 2	40 ± 13

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Size of reflex following drug.

Table 5.59 **Saline 20 μl i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Decrease in MAP (mmHg)	-23 ± 5	-22 ± 5 **(-1±2)**	-21 ± 5 **(-2±2)**	-20 ± 5 **(-3±3)**	-21 ± 5 **(-4±2)**
Increase in R-R interval (ms)	37 ± 7	41 ± 7 **(4±3)**	39 ± 6 **(1±1)**	47 ± 7 **(10±4)**	42 ± 9 **(5±4)**

Table 5.60 **Buspirone 200 μg kg-1 i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Decrease in MAP (mmHg)	-17 ± 2	-14 ± 2 **(-3±1)**	-13 ± 2 **(-5±1)**	-14 ± 2 **(-4±2)**	-14 ± 2 **(-3±2)**
Increase in R-R interval (ms)	40 ± 13	66 ± 22 **(26±15)**	100 ± 27* **(60±17**)**	89 ± 26 **(43±17*)**	83 ± 24 **(37±15*)**

Statistical comparison = saline : buspirone

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Data obtained from anaesthetized rats stimulated with smoke.

Table 5.61 Baseline values, before administration of drug.

Drug	Dose and route	n	Baseline MAP (mmHg)	Baseline R-R interval (ms)	Baseline respiratory rate (Breaths min^{-1})
Saline (Vehicle)	20 µl i.c.	5	75 ± 9	167 ± 6	116 ± 8
Buspirone	200 µg kg^{-1} i.c.	5	74 ± 7	165 ± 5	123 ± 5
(+)8-OH-DPAT	25 µg kg^{-1} i.c.	5	86 ± 4	167 ± 1	124 ± 4
WAY-100635	100 µg kg^{-1} i.c.	4	78 ± 5	155 ± 3	160 ± 29
WAY-100802	50 µg kg^{-1} i.c.	5	75 ± 7	173 ± 3	122 ± 10
(+)8-OH-DPAT	25 µg kg^{-1} i.c.	5	94 ± 3	171 ± 3	114 ± 7
(WAY-100802 pretreated)	(50 µg kg^{-1} i.c.)				
Granisetron	20 µg kg^{-1} i.c.	3	80 ± 7	167 ± 3	127 ± 3
Acidified saline (Vehicle)	20 µl i.c.	4	78 ± 2	166 ± 5	113 ± 11
(-)Pindolol	100 µg kg^{-1} i.c.	4	81 ± 9	164 ± 6	123 ± 8
Atenolol	1 mg kg^{-1} i.v.	10	86 ± 3	148 ± 5	129 ± 5

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Resting baseline values after drug.

Table 5.62 **Saline 20 μl i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	75 ± 9	78 ± 9 **(2±7)**	83 ± 6 **(8±8)**	77 ± 7 **(1±4)**	80 ± 5 **(4±6)**
R-R interval (ms)	167 ± 6	166 ± 5 **(-1±2)**	165 ± 5 **(-2±2)**	166 ± 7 **(-2±5)**	164 ± 5 **(-4±4)**
Phrenic burst rate(bursts min^{-1})	116 ± 8	115 ± 7 **(-1±3)**	111 ± 3 **(-5±6)**	112 ± 6 **(-4±4)**	110 ± 6 **(-6±5)**

Table 5.63 **Buspirone 200 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	74 ± 7	69 ± 8 **(-5±9)**	77 ± 4 **(3±6)**	84 ± 8 **(10±3)**	88 ± 5 **(14±5)**
R-R interval (ms)	167 ± 5	177 ± 6 **(9±2)**	178 ± 7 **(10±6*)**	176 ± 6 **(8±4)**	172 ± 6 **(5±3)**
Phrenic burst rate(bursts min^{-1})	123 ± 5	152 ± 11**(29±9**)**	130 ± 5 **(7±5)**	134 ± 13 **(11±12)**	128 ± 14 **(5±12)**

Statistical comparison = saline : buspirone

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Table 5.64 **(+)8-OH-DPAT 25 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	86 ± 4	80 ± 5 **(-6±6)**	80 ± 5 **(-5±5)**	75 ± 3 **(-11±4)**	73 ± 1 **(-12±4*)**
R-R interval (ms)	167 ± 1	168 ± 4 **(1±4)**	177 ± 4 **(10±4*)**	175 ± 4 **(8±4)**	173 ± 1 **(6±2)**
Phrenic burst rate(bursts min^{-1})	124 ± 4	177 ± 2** **(59±5**)**	134 ± 4* **(10±0)**	152 ± 9** **(28±9**)**	144 ± 10** **(20±9**)**

Statistical comparison = saline : 8-OH-DPAT

Table 5.65 **WAY-100635 100 μg kg^{-1} i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	78 ± 5	61 ± 3 **(-17±3*)**	78 ± 3 **(0±3)**	77 ± 6 **(-1±1)**	77 ± 6 **(-1±2)**
R-R interval (ms)	155 ± 3	154 ± 5 **(6±2)**	153 ± 5 **(4±2)**	151 ± 4 **(1±1)**	152 ± 3 **(3±3)**
Phrenic burst rate(bursts min^{-1})	160 ± 29	108 ± 16 **(-52±15**)**	139 ± 12 **(-21±8)**	164 ± 29* **(4±10)**	159 ± 21* **(-1±6)**

Statistical comparison = saline : WAY-100635

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Table 5.66 **WAY-100802 50 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15
MAP (mmHg)	75 ± 7	91 ± 8 **(16±5)**	85 ± 6 **(10±4)**
R-R interval (ms)	173 ± 3	170 ± 2 **(-2±4)**	170 ± 3 **(-2±2)**
Phrenic burst rate(bursts min^{-1})	122 ± 10	85 ± 6* **(-37±9**)**	126 ± 10 **(4±4)**

Statistical comparison = saline : WAY-100802

Table 5.67 **(+)8-OH-DPAT 25 μg kg^{-1} i.c.** (**WAY-100802 50 μg kg^{-1} i.c.** pretreated 20 minutes previously) n=5.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	94 ± 3	78 ± 7 **(-15±6)**	82 ± 6 **(-11±4)**	83 ± 5 **(-10±5)**	84 ± 5 **(-10±6)**
R-R interval (ms)	171 ± 3	178 ± 5 **(7±3)**	174 ± 5 **(3±3)**	171 ± 5 **(0±2)**	175 ± 5 **(4±3)**
Phrenic burst rate(bursts min^{-1})	114 ± 7	123 ± 13** **(9±11**)**	134 ± 13 **(18±11)**	126 ± 11 **(12±8)**	125 ± 13 **(11±10)**

Statistical comparison = 8-OH-DPAT : 8-OH-DPAT + WAY-100802

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Table 5.68 **Granisetron 20 μg kg-1 i.c. n=3.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	80 ± 7	88 ± 9 **(8±4)**	78 ± 6 **(-2±1)**	79 ± 4 **(-1±3)**	84 ± 8 **(4±3)**
R-R interval (ms)	167 ± 3	168 ± 6 **(1±5)**	165 ± 6 **(-2±4)**	162 ± 4 (-5±3)	164 ± 4 **(-3±2)**
Phrenic burst rate(bursts min^{-1})	127 ± 3	127 ± 13 **(0±10)**	130 ± 6 **(3±3)**	127 ± 15 **(0±12)**	120 ± 6 **(-7±3)**

Statistical comparison = saline : granisetron

Table 5.69 **Acidified saline 20 μl i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	78 ± 2	83 ± 5 **(5±3)**	78 ± 2 **(0±3)**	78 ± 2 **(1±1)**	81 ± 5 **(3±3)**
R-R interval (ms)	166 ± 5	171 ± 6 **(6±1)**	167 ± 5 **(1±1)**	165 ± 6 **(-1±2)**	163 ± 6 **(-3±2)**
Phrenic burst rate(bursts min^{-1})	113 ± 11	111 ± 12 **(-1±1)**	123 ± 10 **(10±4)**	128 ± 9 **(15±6)**	128 ± 6 **(15±6)**

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Table 5.70 **(-)Pindolol 100 μg kg^{-1} i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	81 ± 9	93 ± 11 **(12±4)**	89 ± 6 **(8±7)**	96 ± 6 **(15±7)**	95 ± 7 **(14±8)**
R-R interval (ms)	163 ± 7	170 ± 7 **(6±9)**	168 ± 11 **(4±14)**	166 ± 9 **(3±13)**	171 ± 7 **(8±11)**
Phrenic burst rate(bursts min^{-1})	123 ± 8	68 ± 14* **(-54±14**)**	75 ± 12** **(-48±9**)**	93 ± 16* **(-30±12**)**	108 ± 11 **(-15±13*)**

Statistical comparison = acidified saline : pindolol

Table 5.71 **Atenolol 1 mg kg^{-1} i.v. n=10.**

Time after drug (min)	-5	5	15
MAP (mmHg)	86 ± 3	100 ± 4** **(14±3**)**	91 ± 3 **(6±4)**
R-R interval (ms)	148 ± 5	169 ± 3** **(21±3**)**	168 ± 3** **(20±3**)**
Phrenic burst rate(bursts min^{-1})	129 ± 5	125 ± 5 **(-5±3)**	127 ± 6 **(-2±3)**

Statistical comparison = 5 min before atenolol : atenolol at time indicated

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Table 5.72 Size of reflex before drug.

Drug	Dose and route	n	Increase MAP (mmHg)	Increase R-R interval (ms)	Apnoea duration (s)
Saline (Vehicle)	20 µl i.c.	5	48 ± 7	70 ± 50	6 ± 1
Buspirone	200 µg kg^{-1} i.c.	5	38 ± 3	47 ± 14	7 ± 1
(+)8-OH-DPAT	25 µg kg^{-1} i.c.	5	34 ± 4	45 ± 6	7 ± 1
WAY-100635	100 µg kg^{-1} i.c.	4	35 ± 3	19 ± 6	6 ± 1
WAY-100802	50 µg kg^{-1} i.c.	5	42 ± 3	27 ± 1	5 ± 1
(+)8-OH-DPAT	25 µg kg^{-1} i.c.	5	44 ± 6	30 ± 4	7 ± 1
(WAY-100802 pretreated)	(50 µg kg^{-1} i.c.)				
Granisetron	20 µg kg^{-1} i.c.	3	37 ± 9	32 ± 8	9 ± 5
Acidified saline (Vehicle)	20 µl i.c.	4	40 ± 6	34 ± 6	6 ± 1
(-)Pindolol	100 µg kg^{-1} i.c.	4	32 ± 11	34 ± 1	5 ± 0
Atenolol	1 mg kg^{-1} i.v.	10	59 ± 3	44 ± 7	5 ± 0

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Size of reflex after drug.

Table 5.73 **Saline 20 μl i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	47 ± 6	46 ± 10 **(-1±5)**	46 ± 7 **(-1±4)**	52 ± 7 **(5±5)**	48 ± 7 **(0±3)**
Peak R-R increase (ms)	25 ± 4	20 ± 3 **(-5±2)**	32 ± 6 **(7±9)**	37 ± 12 **(12±10)**	23 ± 4 **(-2±2)**
3s R-R increase (ms)	16 ± 2	12 ± 1 **(-5±2)**	14 ± 3 **(-2±3)**	17 ± 3 **(1±3)**	14 ± 2 **(-3±1)**
Apnoea duration (s)	6 ± 1	5 ± 0 **(-1±1)**	6 ± 1 **(1±0)**	6 ± 2 **(0±1)**	6 ± 1 **(0±1)**

Table 5.74 **Buspirone 200 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	38 ± 3	28 ± 5 **(-10±4)**	31 ± 4 **(-7±6)**	30 ± 8* **(-8±9)**	32 ± 8 **(-6±8)**
Peak R-R increase (ms)	38 ± 15	33 ± 23 **(-6±9)**	12 ± 9 **(-27±8*)**	14 ± 8 **(-25±12*)**	48 ± 33 **(8±19)**
Apnoea duration (s)	7 ± 1	1 ± 1** **(-6±1**)**	2 ± 1** **(-5±1**)**	2 ± 1** **(-5±2**)**	4 ± 1 **(-3±2)**

Statistical comparison = saline : buspirone

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Table 5.75 **(+)8-OH-DPAT 25 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	34 ± 4	27 ± 4* **(-8±5)**	26 ± 5* **(-8±5)**	28 ± 6* **(-6±6)**	26 ± 6* **(-8±5)**
Peak R-R increase (ms)	45 ± 6	13 ± 9 **(-32±8*)**	1 ± 5** **(-43±7**)**	4 ± 6** **(-41±3**)**	9 ± 13 **(-36±12**)**
Apnoea duration (s)	7 ± 1	0 ± 0** **(-6±1**)**	1 ± 1** **(-6±1**)**	0 ± 0** **(-7±2**)**	0 ± 0** **(-7±1**)**

Statistical comparison = saline : 8-OH-DPAT

Table 5.76 **WAY-100635 100 μg kg^{-1} i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	35 ± 3	33 ± 5 **(-2±3)**	29 ± 8 **(-6±6)**	29 ± 5* **(-5±2)**	35 ± 3 **(0±1)**
Peak R-R increase (ms)	19 ± 6	22 ± 8 **(3±2)**	21 ± 5 **(2±2)**	16 ± 6 **(-3±3)**	22 ± 7 **(3±4)**
3s R-R increase (ms)	14 ± 4	14 ± 4 **(0±1)**	15 ± 4 **(1±0)**	12 ± 3 **(-2±3)**	15 ± 5 **(1±1)**
Apnoea duration (s)	6 ± 1	9 ± 3 **(3±1*)**	10 ± 3 **(4±2*)**	9 ± 3 **(3±2)**	10 ± 3 **(4±1*)**

Statistical comparison = saline : WAY-100635

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Table 5.77 **WAY-100802 50 μg kg^{-1} i.c. n=5.**

Time after drug (min)	-5	5	15
Increase MAP (mmHg)	42 ± 3	41 ± 3 **(-1±2)**	45 ± 5 **(3±4)**
Peak R-R increase (ms)	27 ± 1	19 ± 2 **(-7±2)**	32 ± 4 **(6±5)**
Apnoea duration (s)	5 ± 1	7 ± 2 **(2±1*)**	7 ± 2 **(2±1)**

Statistical comparison = saline : WAY-100802

Table 5.78 **(+)8-OH-DPAT 25 μg kg^{-1} i.c.** (**WAY-100802 50 μg kg^{-1} i.c.** pretreated 20 minutes previously), n=5.

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	44 ± 6	50 ± 8** **(7±4*)**	49 ± 6** **(6±3*)**	48 ± 5* **(5±6)**	47 ± 5 **(4±5)**
Peak R-R increase (ms)	30 ± 4	32 ± 8 **(3±7**)**	31 ± 6** **(2±5**)**	28 ± 1* **(-2±3**)**	26 ± 3 **(-3±5**)**
Apnoea duration (s)	7 ± 1	6 ± 1** **(-2±1**)**	6 ± 1** **(-2±1**)**	6 ± 1** **(-1±1**)**	6 ± 1** **(-1±1**)**

Statistical comparison = 8-OH-DPAT : 8-OH-DPAT + WAY-100802

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Table 5.79 **Granisetron 20 μg kg^{-1} i.c. n=3.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	37 ± 9	32 ± 8 **(-5±4)**	44 ± 4 **(7±7)**	39 ± 3 **(2±7)**	32 ± 6 **(-6±4)**
Peak R-R increase (ms)	32 ± 8	25 ± 5 **(-7±3)**	24 ± 5 **(-8±6)**	22 ± 6 **(-10±3)**	25 ± 6 **(-7±2)**
Apnoea duration (s)	9 ± 5	8 ± 1 **(-1±3)**	8 ± 2 **(-1±3)**	9 ± 3 **(0±2)**	8 ± 3 **(-1±2)**

Statistical comparison = saline : granisetron

Table 5.80 **Acidified saline 20 μl i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	40 ± 6	34 ± 5 **(-6±3)**	39 ± 6 **(0±2)**	35 ± 6 **(-4±3)**	33 ± 8 **(-6±7)**
Peak R-R increase (ms)	34 ± 6	34 ± 12 **(0±8)**	36 ± 8 **(2±3)**	34 ± 4 **(1±10)**	33 ± 6 **(-1±11)**
Apnoea duration (s)	6 ± 1	8 ± 4 **(2±3)**	8 ± 2 (2±2)	9 ± 3 **(3±3)**	8 ± 3 **(2±4)**

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Table 5.81 **(-)Pindolol 100 μg kg-1 i.c. n=4.**

Time after drug (min)	-5	5	15	25	35
Increase MAP (mmHg)	32 ± 5	8 ± 9** **(-25±7*)**	8 ± 7** **(-24±7**)**	7 ± 9** **(-26±7**)**	15 ± 4 **(-17±4)**
Peak R-R increase (ms)	34 ± 1	13 ± 6* **(-21±6*)**	12 ± 5** **(-22±6*)**	13 ± 4* **(-21±5*)**	16 ± 5 **(-18±6)**
Apnoea duration (s)	5 ± 0	3 ± 1 **(-2±1)**	3 ± 1 **(-1±1)**	3 ± 1* **(-2±1)**	3 ± 1 **(-2±1)**

Statistical comparison = acidified saline : pindolol

Table 5.82 **Atenolol 1 mg kg-1 i.v. n=10.**

Time after drug (min)	-5	5	15
Increase MAP (mmHg)	59 ± 3	35 ± 3** **(-24±1**)**	41 ± 3** **(-17±2**)**
Peak R-R increase (ms)	44 ± 7	29 ± 2 **(-15±7)**	30 ± 6 **(-14±5)**
Apnoea duration (s)	5 ± 0	5 ± 0 **(-1±1)**	5 ± 1 **(0±1)**

Statistical comparison = 5 min before atenolol : atenolol at time indicated

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Data obtained from anaesthetized rats, stimulated with phenyl biguanide administered into the right atrium.

WAY-100635 200 μg kg^{-1} i.c. n=4.

Table 5.83 Baseline values before PBG challenge.

Time after drug (min)	-5	5	15	25	35
MAP (mmHg)	74 ± 9	67 ± 9 **(-7±2)**	76 ± 11 **(2±6)**	80 ± 16 **(5±8)**	82 ± 16 **(7±8)**
R-R interval (ms)	174 ± 2	175 ± 2 **(1±1)**	180 ± 2 **(6±3)**	178 ± 1 **(4±2)**	175 ± 3 **(1±3)**

No statistical comparison

Table 5.84 Size of reflex following drug.

Time after drug (min)	-5	5	15	25	35
Decrease MAP (mmHg)	-22 ± 4	-8 ± 2 **(-14±4)**	-19 ± 2 **(-4±3)**	-25 ± 5 **(3±2)**	-22 ± 6 **(-1±3)**
Increase R-R interval (ms)	35 ± 10	27 ± 4 **(-8±10)**	47 ± 18 **(12±10)**	46 ± 13 **(11±4)**	33 ± 8 **(-2±3)**

No statistical comparison

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Synchronized ventilation of the upper and lower airways of the anaesthetized rat using a phrenic-triggered respirator

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This ventilator is designed to allow airflow through the upper and lower airways to be timed both with respect to each other and phrenic nerve activity (Fig. 1*A*). Integrated phrenic nerve activity is used to trigger a solenoid-controlled valve which directs airflow into the lungs whilst sucking air through the upper airways, allowing nose and/or mouth breathing to be simulated (Fig. 1*B*). The design incorporates the concept of a synchronous upper airway ventilator designed by Nolan *et al.* (1991) and a phrenic-triggered lower airway ventilator used by Cohen *et al.* (1984).

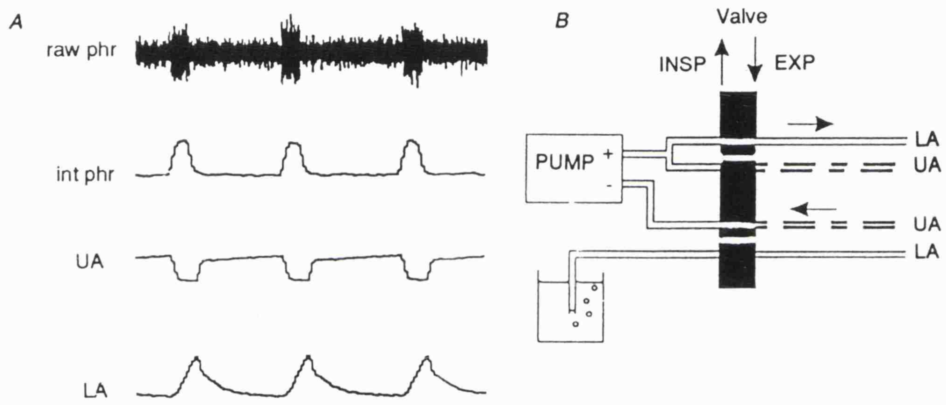

Fig. 1. *A*, the relationships between phrenic nerve activity (raw phr), integrated phrenic activity (int phr) and the pressures in upper (UA) and lower (LA) airways. *B*, during inspiration (INSP) the valve directs the positive pressure from the PUMP to the LA and sucks air through the UA. This is reversed during expiration (EXP).

This ventilator allows physiological and pharmacological stimuli to be applied independently to the upper and lower airways. Central recordings can be made, allowing reflex pathways involved in cardiorespiratory responses to be studied.

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472P EVIDENCE THAT BUSPIRONE POTENTIATES THE VAGAL BRADYCARDIA INDUCED BY UPPER AIRWAY STIMULATION IN ANAESTHETISED RABBITS

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5-HT_{1A} receptors modulate the excitability of cardiac vagal motoneurones (CVM's) and consistent with this, in anaesthetized rabbits, the activation of CVM's by the "diving response" is potentiated by buspirone (Futuro-Neto *et al.*, 1993). The "diving response" is due to the interaction of upper airway receptors and chemoreceptors (Daly & Angell-James, 1979) and also causes a rise in blood pressure and an apnoea. Experiments were carried out to determine whether activation of CVM's (by analyzing R-R interval at 3s) and the apnoea evoked by stimulation of upper airway receptors alone are potentiated by buspirone and to determine (by analyzing peak R-R interval) if peripheral chemoreceptors contribute to this by repeating these experiments in hyperoxic animals so that Po_2 is maintained above normal during the apnoea.

Rabbits were anaesthetized with urethane i.v. 1.5g kg^{-1}. Simultaneous recordings were made of blood pressure, renal (RNA) and phrenic nerve activity (PNA; Shepheard et al., 1990). ECG was also recorded from which changes in R-R interval were calculated and arterial blood gases were monitored. Bi-directional tracheal cannulation was performed to allow either spontaneous ventilation with air or oxygen enriched air and the independent delivery of herbal cigarette smoke to the upper airways. Smoke volume (10-50 ml) was chosen to produce a sub maximal bradycardia. Atenolol (1mg kg^{-1}) was then administered i.v. and the smoke challenge repeated every 10 min until a stable bradycardia was observed. 5 min after the stable smoke challenge saline or buspirone (200μg kg^{-1}) was administered i.c. (20 μl over 20s) and the smoke challenges repeated for another 25 min. Time matched comparisons of buspirone induced changes in the reflex with that of vehicle were made by ANOVA.

15 min after the administration of buspirone (n=4) there was a significant ($P < 0.05$) potentiation of the changes in both 3s and peak R-R interval by 21 ± 5 ms and 61 ± 23 ms (mean ± s.e.mean) compared with that of saline (n=4) 1 ± 2 and -6 ± 10 ms. The duration of the apnoea was unaffected, 18 ± 7 s compared to 16 ± 3 s after saline. In addition, buspirone failed to affect the increase in RNA ($412 \pm 107\%$ c.f. $306 \pm 55\%$) or BP (23 ± 4 c.f. 19 ± 3 mmHg). In hyperoxic animals (Po_2 180-200 mmHg which never fell below 120 mmHg by the end of the apnoea) buspirone (n=4) again significantly potentiated the changes in 3s and peak R-R interval by 38 ± 11 ms and 103 ± 4 ms compared with that of saline (n=4). The duration of the apnoea was again unaffected 18 ± 7s compared to 17 ± 4s for saline. These results indicate that 5-HT_{1A} receptors are involved in the reflex activation of CVM's by stimulation of the upper-airways and that this does not involve peripheral chemoreceptors.

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Opposite effects of central 5-HT_{1A} receptors on reflex activation of cardiac vagal motoneurones in anaesthetized rabbits and rats

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Activation of cardiac vagal motoneurones (CVMs) by stimulating cardiopulmonary receptors with phenylbiguanide (PBG) in rats is inhibited (Bogle *et al.* 1990) whilst that evoked by stimulation of upper airway receptors with smoke in rabbits is potentiated (Futuro-Neto *et al.* 1993) by the central administration of 5-HT_{1A} receptor antagonists. This may suggest that 5-HT receptors have opposing actions on the reflex pathways activated by stimulation of the two groups of receptor, or may reflect variation between the species. This was tested in the present study.

Rabbits and rats were anaesthetized with urethane (1·5 g kg^{-1} I.V.) and pretreated with atenolol (1 mg kg^{-1} I.V.). Recordings were made of arterial blood pressure and ECG, and in rabbits renal (RNA) and phrenic nerve activity (Shepheard *et al.* 1990). R–R interval was calculated from the ECG. A volume of smoke or a dose of PBG (10–20 μg kg^{-1}, right atrium) was chosen which evoked a sub-maximal bradycardia. The challenge was repeated at 10 min intervals until a stable response was observed. Five minutes after stability was achieved, either saline or a 5-HT_{1A} antagonist buspirone (200 μg kg^{-1}) was administered I.C. (20 μl volume, over 20 s) and the challenge was then repeated. Time-matched comparisons of drug-induced changes with those of saline were made by ANOVA.

In rabbits, the smoke-induced increase in R–R interval was significantly potentiated by 61 ± 23 ms 15 min after buspirone I.C. ($n = 4$), compared with -6 ± 10 ms 15 min after saline I.C. ($n = 4$). The duration of the evoked apnoea (18 ± 7 s) was unaffected as were the increases in RNA (412 ± 107 %) and mean arterial blood pressure (MAP; 23 ± 4 mmHg). In contrast, in rats, the smoke-induced increase in R–R interval was significantly inhibited by 33 ± 8 ms following buspirone administration ($n = 4$). Again, there was no significant effect on the observed increase in MAP (29 ± 5 mmHg). Finally, in rabbits, the PBG induced increase in R–R interval was potentiated by 64 ± 19 ms ($n = 4$) following buspirone administration. Since buspirone had no significant effect on baseline R–R interval in either rats or rabbits these results suggest that the opposite effect of 5-HT_{1A} antagonists on the changes in R–R interval evoked by stimulating cardiopulmonary and upper airway receptors in rats and rabbits reflects a species difference rather than a different action of 5-HT receptors on the reflex pathway.

S.B.D. is a BHF PhD student. We thank Mr S. Wilkinson for technical support.

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The role of central 5-HT_3 receptors in the modulation of the response to upper airway stimulation in the anaesthetized rabbit.

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Stimulation of the upper airways with herbal cigarette smoke in anaesthetized rabbits evokes a vagal bradycardia, apnoea, renal sympathoexcitation and pressor response. It has been shown that the bradycardia can be modulated by 5-HT_{1A} ligands (Futuro-Neto et al., 1993). As 5-HT_3 receptors are also present in brainstem areas containing terminals of trigeminal and vagal afferents (Waeber et al., 1988), experiments were carried out to examine the effects of the 5-HT_3 antagonist granisetron on the cardio-respiratory responses to upper airway stimulation.

Rabbits were anaesthetized with urethane (1.5g kg^{-1}i.v.) and simultaneous recordings made of mean blood pressure (MAP), renal (RNA) and phrenic nerve activities. ECG was recorded from which R-R intervals were calculated. Bi-directional tracheal cannulation was performed below the larynx to allow spontaneous respiration with room air and independent delivery of smoke to the upper airways. A smoke volume (10-50 ml) which produced a sub-maximal bradycardia was chosen, atenolol (1 mg kg^{-1}i.v.) administered and the smoke challenges repeated every 10 min until a stable bradycardia was observed. After 5 min, granisetron (20 μg kg^{-1}; i.c. or i.v.) or saline i.c. (all 20 μl over 20s) was administered and smoke challenges repeated for another 35 min. Time matched comparisons of changes induced in the reflex by granisetron i.c. with those of vehicle and granisetron i.v. were made by ANOVA.

Baseline MAP, R-R interval and respiratory rate were unaffected by granisetron but there was a small but significant increase in baseline RNA. 15 min after administration of granisetron i.c. (n=5) there was a significant ($p<0.05$) inhibition of the smoke-induced increase in R-R interval and apnoea duration (Table 1).

These results demonstrate that the vagal bradycardia and apnoea but not the sympathoexcitation evoked by stimulation of the upper airway involve the activation of central 5-HT_3 receptors.
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Drug & Route	R-R interval (ms)	Apnoea (s)	MAP (mmHg)	RNA (%)
Granisetron i.c.	-121±55*	-7±3*	-3±1	9±13
Granisetron i.v.	14±18	-1±3	-2±3	0±7
Saline i.c.	-7±11	-1±2	0±1	-6±6

TABLE 1. Changes in smoke induced responses in anaesthetized rabbits 15 min following drug administration. n=5 for each group.

BRAINSTEM 5-HT_{1A} AND 5-HT_{1D} RECEPTORS MODULATE THE CARDIORESPIRATORY RESPONSES TO UPPER AIRWAY STIMULATION IN RABBITS. A.G. Ramage,* S. Dando & D. Jordan.#
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Previous studies have demonstrated that central application of buspirone, a partial agonist at 5-HT_{1A} receptors, potentiates the bradycardia evoked by upper airway stimulation in rabbits (Futuro-Neto et al., Brain Res., **629**, 349-354, 1993). The present study compared the effects of 5-HT_{1A} and 5-HT_{1D} receptors on the cardiorespiratory responses evoked by stimulating upper airway receptors.

Studies were carried out in urethane-anaesthetized, atenolol pretreated rabbits. Bi-directional tracheal cannulation allowed ventilation of the lungs and independent delivery of cigarette smoke to the upper airways. Recordings were made of baseline and reflexly-evoked changes in mean arterial blood pressure (BP), ECG, renal (RNA) and phrenic nerve activities. Buspirone ($200\mu g\ kg^{-1}$ i.c., n=5) significantly decreased BP and RNA, increased phrenic rate and R-R interval, and potentiated the bradycardia evoked by a smoke challenge. These effects were abolished by pretreatment with WAY-100635 ($100\mu g\ kg^{-1}$ i.v.), a 5-HT_{1A} receptor antagonist. In contrast, following pretreatment with WAY-100635, the 5-HT_{1D} receptor agonist sumatriptan ($50\mu g\ kg^{-1}$ i.c.) significantly increased baseline BP, phrenic rate and RNA and attenuated the bradycardia, depressor response and apnoea evoked by smoke.

Theses data are consistent with the view that brainstem 5-HT_{1A} and 5-HT_{1D} receptors have modulatory roles in cardiorespiratory integration.

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